Vol. 29, No. 2 S. P. WILTSHIRE. May, 1942

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF APPLIED BIOLOGISTS

BY

W. B. BRIERLEY

AND

C. T. GIMINGHAM

PUBLICATIONS COMMITTEE

G. C. AINSWORTH R. W. MARSH

W. E. H. HODSON F. R. PETHERBRIDGE

W. C. MOORE

CAMBRIDGE UNIVERSITY PRESS

LONDON: BENTLEY HOUSE

CHICAGO: The University of Chicago Press
(Agents for the United States)

BOMBAY, CALCUTTA, MADRAS: Macmillan

All rights reserved

PRINTED IN GREAT BRITAIN

The Association of Applied Biologists

President
H. MARTIN, D.Sc., F.I.C.

Vice-Presidents
Miss W. E. BRENCHLEY, D.Sc. G. E. BLACKMAN, M.A.

Hon. Treasurer
W. C. MOORE, M.A.
Plant Pathological Laboratory,
Milton Road, Harpenden, Herts.

Hon. Editor (General and Botanical)
Prof. W. B. BRIERLEY, D.Sc.
University of Reading,
Berks.

Hon. Editor (Zoological)
C. T. GIMINGHAM, B.Sc., F.I.C.
Plant Pathological Laboratory,
Milton Road, Harpenden, Herts.

Hon. Secretary (General and Botanical)
W. P. K. FINDLAY, D.Sc.
Forest Products Research Laboratory
Princes Risborough, Bucks.

Hon. Secretary (Zoological)
H. F. BARNES, M.A., Ph.D.
Rothamsted Experimental Station,
Harpenden, Herts.

Council

G. C. AINSWORTH, Ph.D.
G. E. BLACKMAN, M.A.
Miss W. E. BRENCHLEY, D.Sc.
D. E. GREEN, M.Sc.
W. E. H. HODSON, D.I.C.
S. E. JACOBS, Ph.D.
O. B. LEAN, B.Sc.
E. A. PARKIN, Ph.D.
C. POTTER, Ph.D.
W. STEER, B.A.
I. THOMAS, Ph.D.
W. A. R. DILLON WESTON, M.A., Ph.D.

CONTENTS OF Vol. 29, No. 2

PAGE

1. The nutrition of spinach. By R. M. Woodman 97
2. The effectiveness of certain strains of the soya-bean nodule organism when associated with different varieties of the host plant. By J. Boyes and G. Bond 103
3. Investigations on potato root eelworm, *Heterodera rostochiensis* Wollenweber. On the cyst population of a field over a series of years. By Herbert W. Miles, D.Sc., Ph.D. and Mary Miles, M.Sc. . 109
4. Observations on the biology of the carrot fly (*Psila rosae* Fab.): assembling and oviposition. By F. T. Baker, F.R.E.S., I. E. Ketteringham, N.D.A., S. P. V. Bray, B.Sc. and J. H. White, B.Sc. (With 6 Text-figures) 115
5. Studies on the mechanism of fungicidal action. II. Elements of the theory of variability. By A. F. Parker-Rhodes, B.A. 126
6. Studies on the mechanism of fungicidal action. III. Sulphur. By A. F. Parker-Rhodes, B.A. (With 2 Text-figures) 136
7. Proceedings of the Association of Applied Biologists: 10 October 1941. Symposium on wireworm investigations:
   - Investigations on wireworms: introductory. By J. C. F. Fryer, M.A. 144
   - Wireworms and crop production. By S. G. Jary, B.A. 150
   - Statistical problems in field sampling for wireworms. By F. Yates, Sc.D., and D. J. Finney, M.A. (With 3 Text-figures) 156
   - Observations on some factors influencing growth in wireworms of the genus *Agriotes* Esch. By A. C. Evans, Ph.D., and H. C. Gough, Ph.D. (With 4 Text-figures) 168
   - Wireworms and agriculture, with special reference to *Agriotes obscurus* L. By Herbert W. Miles, D.Sc., Ph.D. 176
   - Observations on the biology of *Agriotes obscurus* L. I. The adult insect. By Morris Cohen, M.Sc., Ph.D. (With 8 Text-figures) 181
8. Proceedings of the Association of Applied Biologists: 5 December 1941. A. Discussion on some problems in war-time horticulture with particular reference to vegetable production. Problems in vegetable production: introductory. By H. V. Taylor, D.Sc. Some problems of the glasshouse industry in war time. By W. F. Bewley, C.B.E., D.Sc. Some aspects of the nutrition of vegetable crops. By T. Wallace, D.Sc. The control of weeds in onions. By G. E. Blackman, M.A. B. Some aspects of tree surgery in Canada. By Chester Cook 197
9. Report of the Council of the Association of Applied Biologists for the year 1941 209
10. Report of the Hon. Treasurer for the year ended 31 December 1941 211

THE NUTRITION OF SPINACH

BY R. M. WOODMAN
Horticultural Research Station, Cambridge University

THE effects of varying the supply of certain nutrients on Clucas's New Giant Leaved Spinach are described in this paper.

EXPERIMENTAL

General methods have been described previously (Woodman, 1940). The nitrogen experiments were carried out in 9 in. waxed plant-pots, each of which held 13 lb. of the standard sand used in this series of researches, and the potassium, boron, and phosphorus experiments, for reasons discussed before (Woodman, 1941*a*), in glazed culture pots, each holding 46 lb. of the sand. Concentrations of elements present in the nutrient solution are given in Table 1. The figures in heavy type show the variations for N, P, K, and B during an experiment, and the necessary accompanying variations in Na and S. The salts used have already been described (Woodman, 1939).

THE DATA OBTAINED

Descriptions of a table similar to Tables 2–4, of the eleven numerical values obtained, and of methods of interpreting the results, have also been discussed previously (Woodman, 1940). The symbols S, SS, SSS, and NS, indicate that differences between the results for the value under discussion are significant at the 5, 1, or 0·1 points, by the z test, or are not significant, respectively.

OBSERVATIONS, AND DISCUSSION OF RESULTS

Nitrogen experiment

Sixteen replications of a randomized block of seven treatments, A–G, Table 1, were used, making 112 cultures in all. Seed sown, 13 Aug. 1940; germinated, 20 Aug.; singled to one culture per pot, 27 Aug.; and harvested, 16 Oct. 1940. Each pot received 8·5 l. of the appropriate culture solution in lots of 250 c.c. about every 2 days.

On 17 Sept., all the seedlings were normal. Treatments A–D yielded cultures of the same size, but E–G gave smaller plants. At harvest, the three treatments A–C yielded dark, uniform green tops of about the same size. The roots (cut off at sand level) were normal. D gave similar but slightly smaller plants, with normal roots. E yielded slightly lighter coloured cultures, smaller still, with normal roots; two had flower stems forming. Treatment F gave still lighter and smaller plants, with normal roots; three cultures had flower stems forming. With G, very small plants with a tendency to etiolation resulted; the roots were lacking in fibre; three cultures were forming flower stems. The colour, and the occurrence of flower stems, thus tended to bear out the work of Knott (1940), who obtained stockier spinach plants with darker green foliage following heavy applications of sodium nitrate, and a tendency to the formation of less seed-stalks and to a slower rate of seed-stalk elongation.

The summaries of results are in Table 2. The results were significant at the 0·1 % level (SSS) for all the eleven values tabulated. The main point that emerged was that the yields of both fresh and dried tops with A, B, and C were equal (A = B = C); as the nitrogen was further reduced, the weight of tops became less. Nitrogen above a certain amount, therefore, appears to give no response as regards yield of foliage.

TABLE 1. *P.p.m. of elements present in the nutrient solutions*

Nutrient solutions ...	N	P	K	B	S	Na
			Nitrogen experiment			
A	**65·92**	21·84	22·44	0·0681	16·44	**140·70**
B	**32·96**	21·84	22·44	0·0681	16·44	**86·56**
C	**24·72**	21·84	22·44	0·0681	16·44	**73·02**
D	**16·48**	21·84	22·44	0·0681	16·44	**59·48**
E	**8·24**	21·84	22·44	0·0681	16·44	**45·94**
F	**4·12**	21·84	22·44	0·0681	16·44	**39·17**
G	**0·41**	21·84	22·44	0·0681	16·44	**33·08**
			Potassium and boron experiment			
A	32·96	21·84	**44·88**	0·0681	**25·64**	86·56
B	32·96	21·84	**22·44**	**Nil**	**16·44**	86·56
C	32·96	21·84	**22·44**	0·0681	**16·44**	86·56
D	32·96	21·84	**11·22**	0·0681	**11·84**	86·56
E	32·96	21·84	**5·61**	0·0681	**9·54**	86·56
F	32·96	21·84	**2·81**	0·0681	**8·39**	86·56
G	32·96	21·84	**0·28**	0·0681	**7·36**	86·56
			Phosphorus experiment			
A	32·96	**43·68**	22·44	0·0681	16·44	**118·96**
B	32·96	**21·84**	22·44	0·0681	16·44	**86·56**
C	32·96	**16·38**	22·44	0·0681	16·44	**78·46**
D	32·96	**10·92**	22·44	0·0681	16·44	**70·36**
E	32·96	**5·46**	22·44	0·0681	16·44	**62·26**
F	32·96	**2·73**	22·44	0·0681	16·44	**58·21**
G	32·96	**0·27**	22·44	0·0681	16·44	**54·57**

All solutions also contained 9·03 p.p.m. of calcium as chloride, 5·05 p.p.m. of magnesium as sulphate, and 1·01 p.p.m. of iron as ferrous sulphate. The following were the proportions of the minor elements present in all solutions: 0·1847 p.p.m. of Mn as $MnSO_4,4H_2O$; 0·0682 p.p.m. of Zn as the sulphate; 0·0095 p.p.m. of Al as ammonium alum; 0·0164 p.p.m. of the ammonium radicle, NH_4, partly as the alum, and partly as ammonium sulphate; and 0·0153 p.p.m. of Cu as the sulphate. The sulphur combined with these minor elements in the above salts has been neglected in compiling the table.

TABLE 2. *Summaries of results: nitrogen experiment. Weights in g.*

Description of data		Treatment mean for A	B	C	D	E	F	G	Mean of all results	S.E.
Tops, FW	SSS	7·41	6·78	8·25	4·26	2·37	0·93	0·20	4·314	0·5319
				A=B=C>D>E>G; D>E=F; F=G						
Roots, FW	SSS	0·719	0·688	1·135	0·654	0·273	0·107	0·050	0·518	0·0808
				C>A=B=D>E=F=G						
Whole plants, FW	SSS	8·13	7·47	9·39	4·91	2·64	1·04	0·247	4·832	0·5969
				C>B>D>E>G; A=B>D>E=F; A=C; F=G						
Tops, DW	SSS	0·725	0·696	0·828	0·497	0·271	0·128	0·033	0·454	0·0548
				A=B=C>D>E>G; D>E=F; F=G						
Roots, DW	SSS	0·121	0·115	0·174	0·101	0·061	0·025	0·010	0·087	0·0110
				C>A=B=D>E>F=G						
Whole plants, DW	SSS	0·845	0·811	1·002	0·598	0·332	0·154	0·044	0·540	0·0639
				A=B=C>D>E>G; D>E=F; F=G						
Top/root, FW	SSS	10·61	10·78	8·38	7·88	8·93	8·95	3·91	8·49	0·7106
				A=B>C=D>G; C=D=E=F>G; A=B=E=F						
Top/root, DW	SSS	6·10	6·09	5·08	5·48	4·61	5·26	3·30	5·13	0·3983
				A=B=C=D=F>G; A=B>E; C=D=E=F>G						
Tops, % moisture	SSS	90·07	89·40	90·03	88·20	88·25	86·03	82·62	87·80	0·4393
				A=B=C>F>G; A=C>D=E>F; B=D=E						
Roots, % moisture	SSS	82·79	80·63	83·68	83·17	76·83	76·06	79·51	80·38	1·017
				A=C=D>G>F; A=B=C=D>E=F; E=G						
Whole plants, % moisture	SSS	89·56	88·65	89·37	87·64	86·99	84·97	82·10	87·04	0·4398
				A=B=C>E>F>G; A=C>D=E>F; B=D						

Top/root ratios were constant over wide ranges, especially for dry matter. Low values were obtained with G, the least concentration of nitrogen, however. The moisture contents of tops, roots, and whole plants were equal for the first three experiments (A = B = C).

Potassium and boron experiment

Ten replications of seven randomized treatments, A–G, Table 1. Sown, 12 Aug. 1940; germinated, 18 Aug.; singled, 26 Aug.; and harvested, 17 Oct. 1940. Each culture received 14 l. of the appropriate solution in lots of 400 c.c. about every 2 days.

By 17–23 Sept., A had given good cultures. With B, lack of boron, the cultures were smaller, and there was a tendency to yellowish tinting of the foliage ranging to a white amber on the oldest leaves; the younger leaves tended to be wrinkled. C and D gave normal plants, but, with E, a pale biscuit-coloured scorch had appeared on the tips of the oldest leaves. F gave similar symptoms, with a tendency to wrinkling of some of the leaves and turning in of the edges. With G, much smaller plants were obtained, and the oldest leaves were dead; there was a tendency for the leaves to be wrinkled and to turn in at the margins, the turned-in portions being scorched; some of the plants were obviously dying, and this treatment had to be harvested on 23 Sept. to obtain the correct fresh weights.

At harvest, on 17 Oct., cultures with A were normal, both as regards top and roots. Lack of boron, B, gave small plants with chlorotic patches on the older leaves. The buds and new leaves were dead and brown, and the petioles then remaining had thickened and browned. The roots were small and brown, with lack of fibre. C gave normal tops and roots. With D, some plants were normal, but others showed a tendency to marginal scorch. With E also some cultures were normal; but others showed worse marginal scorch than with D, and the affected leaves tended to go limp, lie on the sand, and waste away. F gave much smaller plants, with similar symptoms of potassium deficiency much more developed. The outer leaves wilted, lay on the sand, and rotted, and the inner leaves were small and wrinkled. The roots lacked fibre. One culture with treatment A and one with B showed signs of a flower stem.

Table 3 showed that the two largest amounts of potassium resulted in equal tops (A = C); there was then a decrease on reduction of the potassium. With lack of boron, B, the tops were only equal to those with serious deficiencies of potassium (B = F = G). The dried tops with A were the largest, and those with B, F, and G the smallest (B = F = G). Both the fresh and dried yields of roots did not respond to potassium over a long range (44·88–5·61 p.p.m., A = C = D = E), but were much smaller with serious potassium deficiency, or with lack of boron (B = F = G). The whole plants, fresh and dried, responded in the same manner as the fresh and dried tops, respectively. Top/root ratios for fresh weights were constant over a wide range of potassium (A = C = D = E), but were smaller with less than 5·61 p.p.m. of potassium, or lack of boron (B); the ratios for dry weights were uninfluenced by the concentration of potassium (NS). The moisture contents of tops and whole plants were constant over a wide range of potassium (A = C = D = E), but smaller with amounts less than 5·61 p.p.m., or with lack of boron. There was not even so much variation with the roots, the lowest value being with lack of boron (A = C = D = E = F = G; A = C = F = G > B).

To sum up, it is apparent that a good supply of potassium is necessary for spinach in order to obtain the optimum yield of foliage as well as to avoid scorch.

TABLE 3. *Summaries of results: potassium and boron experiment. Weights in g.*

Description of data		Treatment mean for A	B	C	D	E	F	G	Mean of all results	S.E.
Tops, FW	SSS	11·82	0·96	9·83	7·74	6·93	1·69	0·33	5·61	0·9268
		A>D=E>B=F=G; A=C>E; C=D								
Roots, FW	SSS	0·644	0·142	0·657	0·599	0·456	0·184	0·070	0·393	0·0870
		A=C=D=E>B=F=G								
Whole plants, FW	SSS	12·47	1·10	10·49	8·34	7·39	1·87	0·40	6·01	0·9908
		A>D=E>B=F=G; A=C>E; C=D								
Tops, DW	SSS	1·125	0·161	0·803	0·798	0·671	0·223	0·085	0·552	0·0763
		A>C=D=E>B=F=G								
Roots, DW	SSS	0·087	0·025	0·084	0·087	0·067	0·025	0·009	0·055	0·0096
		A=C=D=E>B=F=G								
Whole plants, DW	SSS	1·212	0·186	0·887	0·886	0·738	0·248	0·094	0·607	0·0824
		A>C=D=E>B=F=G								
Top/root, FW	SSS	19·43	7·16	16·55	15·40	16·39	8·04	5·70	12·67	1·428
		A=C=D=E>B=F=G								
Top/root, DW	NS	14·34	6·41	10·75	11·57	10·55	8·31	10·64	10·37	1·703
Tops, % moisture	SSS	90·08	82·21	91·68	89·20	89·55	82·95	72·91	85·51	0·7523
		A=C=D=E>B=F>G								
Roots, % moisture	S	86·32	80·26	87·12	83·42	84·26	85·89	85·40	84·67	1·377
		A=C=F=G>B; B=D=E; A=C=D=E=F=G								
Whole plants, % moisture	SSS	89·90	82·11	91·40	88·78	89·24	83·81	75·45	85·81	1·542
		A=C=D=E>B=F>G								

Phosphorus experiment

Ten blocks of seven randomized treatments, A–G, Table 1. Seed was sown, 25 Oct. 1940; germinated, 10 Nov.; seedlings singled, 25 Nov.; harvest, 28 Jan. 1941. Each culture received 21·2 l. of the appropriate solution in lots of 400 c.c. about every 2 days.

By 27 Nov. 1940, the seedlings with F and G, the least quantities of phosphorus, were darker green. There was then a tendency for the plants to be smaller with decrease in phosphorus.

At harvest, on 28 Jan. 1941, cultures with A had light green foliage with a tendency to be mottled and chlorotic; some older leaves had patches of greyish white scorch, evidently due to excess phosphorus. The roots were normal. B gave similar plants, without scorch, however. Plants with C were of a darker and more uniform green, with normal roots. E yielded the largest plants, of healthy appearance, and with normal roots; and the F cultures were similar. With G there was a great reduction in size; the plants were normal in colour, except for the oldest leaves, which tended to turn yellow and die. There were no flower stems with any treatment, possibly because of the period of the year.

The quantitative data in Table 4 demonstrated that at this time of the year the amount of growth was small. Nevertheless, all the six sets of results for yield were very strongly significant (SSS).

The comparisons E>A=B=C=D=F>G for the three series of fresh yields, and the somewhat similar ones for the dried yields, seem to be characteristic of certain vegetables grown in sand culture. Thus with turnips (Woodman, 1941*a*) there was a similar low optimum value of phosphorus, while with spring cabbage (Woodman, 1941*b*), no response was obtained over a wide range of phosphorus, a low value giving a yield equal to the

highest concentration employed. With these two vegetables also, as here to a lesser extent, the decreases in yield following further reduction of the phosphorus were abrupt and great. On the other hand, lettuce showed no such behaviour, this vegetable responding regularly and well to increases in the phosphorus supplied (Woodman, 1939).

TABLE 4. *Summaries of results: phosphorus experiment. Weights in g.*

Description of data		Treatment mean for A	B	C	D	E	F	G	Mean of all results	S.E.
Tops, FW	SSS	1·96	1·71	1·83	1·62	2·98	1·83	0·20	1·734	0·1925
		E>A=B=C=D=F>G								
Roots, FW	SSS	0·247	0·251	0·249	0·199	0·366	0·218	0·039	0·224	0·0249
		E>A=B=C=D=F>G								
Whole plants, FW	SSS	2·205	1·962	2·083	1·823	3·344	2·047	0·242	1·958	0·2095
		E>A=B=C=D=F>G								
Tops, DW	SSS	0·264	0·228	0·237	0·194	0·329	0·237	0·053	0·220	0·0205
		E>A=B=C=F>G; A>D; B=C=D=F>G								
Roots, DW	SSS	0·048	0·039	0·037	0·032	0·051	0·027	0·006	0·034	0·0047
		A=E>D=F>G; A=B=C=E; B=C=D=F>G								
Whole plants, DW	SSS	0·312	0·267	0·275	0·226	0·380	0·264	0·059	0·255	0·0223
		E>A=B=C=F>G; A>D; B=C=D=F>G								
Top/root, FW	SS	8·78	6·85	7·51	8·46	8·28	8·83	5·10	7·684	0·7269
		A=C=D=E=F>G; A=B=C=D=E=F; B=G								
Top/root, DW	NS	6·99	5·93	6·41	6·39	6·86	9·38	8·23	7·17	0·9260
Tops, % moisture	SSS	86·15	85·33	86·87	87·88	88·67	86·82	74·76	85·21	1·192
		A=B=C=D=E=F>G								
Roots, % moisture	SS	80·86	83·41	84·60	83·76	85·98	87·69	83·87	84·31	1·044
		F>A=B=D=G; C=E=F>A; B=C=D=E=G								
Whole plants, % moisture	SSS	85·55	85·12	86·60	87·47	88·37	86·93	76·36	85·20	1·092
		A=B=C=D=E=F>G								

Top/root ratios for the fresh yields were quite even, except for G, which was smaller, while the dry-weight ones were not significant (NS). The moisture contents (cf., especially, the tops and whole plants), were also remarkably even; here again the lowest amount of phosphorus, treatment G, was responsible for the lowest moisture content.

SUMMARY

A reduction in the supply of nitrogen as sodium nitrate to spinach grown in sand culture did not at first, over the long range 65·92–24·72 p.p.m. of nitrogen, lead to any reduction in the yield of foliage, and therefore nitrogen appears to be unnecessary for yield above a certain optimum amount; further reduction in the supply led to diminished yields. There was some evidence to show, however, that large amounts of nitrogen tended to retard flowering and to give a good colour. Lack of boron resulted in small plants on which the buds and new leaves died. Deficiency in potassium caused a small plant, scorch, and limpness and death of the leaves; potassium encouraged the yield of foliage. Reduction of phosphorus to 5·46 p.p.m. gave an optimum yield of healthy foliage, but reduction below this led to decreased yields.

I thank Mr J. N. Leonard for his help in these experiments.

REFERENCES

KNOTT, J. E. (1940). Effect of nitrate of soda on the response of spinach to length of day. *Plant Physiol.* **15**, 146–8.

WOODMAN, R. M. (1939). Phosphate deficiency and yield tests on sand cultures of May King lettuce. *J. agric. Sci.* **29**, 229–49.

—— (1940). The nutrition of lettuces grown as sand cultures under glass. *Ann. appl. Biol.* **27**, 5–16.

—— (1941*a*). The nutrition of turnips. *Ann. appl. Biol.* **28**, 1–7.

—— (1941*b*). The nutrition of the spring cabbage. *Ann. appl. Biol.* **28**, 181–8.

(*Received* 6 *November* 1941)

THE EFFECTIVENESS OF CERTAIN STRAINS OF THE SOYA-BEAN NODULE ORGANISM WHEN ASSOCIATED WITH DIFFERENT VARIETIES OF THE HOST PLANT

By J. BOYES

Department of Botany, Royal Technical College, Glasgow

and G. BOND

Department of Botany, University of Glasgow

In respect of a particular cross-inoculation group of leguminous plants, many different strains of the nodule organism exist, and though these may all produce abundant nodulation they vary greatly in their ability to benefit a given host plant. Chen & Thornton (1940) conclude that the relatively insignificant fixation of nitrogen produced by so-called ineffective strains of the organism is not due to any inherent defect in the nitrogen-fixing mechanism of these strains; on the contrary, their results indicate that fixation of nitrogen by unit of bacterial mass in unit time is of the same order for ineffective strains as for effective ones. The amount of bacterial tissue developed is, however, much smaller, and its duration shorter, in the case of ineffective strains, leading to the conclusion that ineffectiveness arises because the host tissues provide a less congenial environment for ineffective strains than they do for effective types. From further experiments, Chen *et al.* (1940) concluded that as a result of infection, soluble substances affecting the growth of the bacteria are formed within the host tissues; in the case of an effective strain these products favour bacterial growth, while in that of an ineffective strain they hinder the growth.

Other evidence that ineffectiveness is not necessarily due to inherent defects in the nitrogen-fixing mechanism of bacterial strains is provided by observations by various investigators that while a given strain may be ineffective when associated with one host plant, it may be effective with another. This evidence also suggests that host plant factors to some extent determine the level of effectiveness of an associated bacterial strain, as concluded by Strong (1940). Earlier demonstrations of such variation in effectiveness were reviewed by Fred *et al.* (1932), and later ones by Wilson (1940). Many of these experiments involved relatively large taxonomic differences in the host plants employed, viz. different species of a given genus, or different genera within a given cross-inoculation group, but in a number of cases merely changing the variety of a host plant was found to modify the effectiveness of associated bacterial strains.

The observations presented in this paper concern the influence of varietal changes in host plant upon the effectiveness of different strains of the soya-bean nodule organism. Of the four varieties of soya bean used in the present work, three have been found to be in some degree suited for cultivation in Great Britain.

Materials and methods

In these experiments, which were carried out in the Botany Department, University of Glasgow, the plants were grown in sand free of available nitrogen, contained in glazed jars each holding 3·5 kg. of sand. After the addition of 500 c.c. of a nitrogen-free form of Hiltner's culture solution plus micro-nutrients, each pot of sand was autoclaved for 3 hr. at 20 lb. pressure.

The following varieties of soya bean were used: 'Manchu', 'Brown C', 'Green Jap', and 'Black O'. Seeds of the first two varieties were kindly provided by the United States Department of Agriculture, Washington, and by the National Institute of Agricultural Botany respectively. Seeds of the second two varieties were purchased from Messrs Fordson Estates, Boreham. The Manchu variety is widely grown in the United States of America, while Brown C and Green Jap have been found to be in some degree suited for seed production in Great Britain. The variety Black O has also been grown here to some extent.

Sound seeds of selected weight were utilized, the maximum range of seed weight employed being 10 mg. for a particular variety. The seeds were initially surface-sterilized.

Four strains of the soya-bean nodule organism were used for inoculation, namely, strains 9, 17, 505, and 507 from the collection in the Department of Agricultural Bacteriology, University of Wisconsin; the authors wish to thank Dr I. L. Baldwin for these. With North American conditions of growth and varieties of host plant these strains are of varying efficiency, nos. 505, 9 and 17 being regarded as effective though in different degrees, while no. 507 is ineffective. Prior to being sown the seeds were dipped into inocula prepared from yeast-mannitol-agar slopes of the organisms. Sowing was effected during May. A slight excess of seeds was sown in each pot and after germination a reduction to the desired number of uniform seedlings was made.

The plants were grown in a series of compartments constructed from wooden frames covered with transparent material (Windolite), occupying a long bench in the greenhouse receiving uniform lighting. The compartments were open above and were 2 ft. high, the front section being removable to allow of access to the plants. All the pots infected with a given strain of the nodule organism were kept in one compartment. The arrangement of the pots within each compartment was frequently changed in order to equalize further the incidence of light. On numerous previous occasions it has been found that under the conditions of experiment obtaining in this department, lateral protection of this type is adequate to prevent cross-infection of leguminous cultures. Further proof of this is provided for the present experiment by the fact that uninoculated control plants, grown in a fifth compartment and treated like the other plants apart from the matter of inoculation, remained free of infection. It will, however, be pointed out later that in the case of two inoculated pots of the 1938 experiment there was clear evidence of cross-infection.

Constant moisture content (12·5% on the weight of dry sand) was maintained by the frequent addition of sterile distilled water or culture solution to bring the pots to an appropriate weight. Leaves falling from the plants in the later stages of growth were collected and stored over formalin, and were subsequently added to the rest of the plant material at harvest. Harvesting was effected at various dates in August and September, all the plants of a given variety being harvested together at a stage in which the pods were fully formed though still green. The contents of each pot were tipped out, and after the roots had been washed free of sand the plants were cut into small portions, dried to constant weight at 95° C., quickly pulverized in a mill, and stored for analysis. Triplicate samples of the dry matter from each pot of plants were taken for estimation of total nitrogen by the Kjeldahl process.

Data

The primary data for fixation of nitrogen are presented in Table 1. The figures for fixation represent the final nitrogen contents of the plants minus the amount of nitrogen contained in the original seeds, ascertained by analysis of similar seeds. The alternative method of computing fixation, namely, by deducting from the nitrogen content of the inoculated plants that of uninoculated control plants, is in general more satisfactory. Here, however, it was known from previous experience that uninoculated soya-bean plants after several months' growth under the conditions of the present experiments show a nitrogen content which is very close to that of the original seeds, though there is a slight tendency to loss of nitrogen (Bond, 1936). Other trials (Bond, 1938) indicate that under the conditions prevailing in the present experiments there is no loss of nitrogen by excretion from nodulated soya-bean plants.

All the plants infected with strain 507 showed signs of extreme nitrogen deficiency (the exception provided by pots 1 and 2 of Manchu beans in the 1938 experiment is considered

below), and it is obvious that although this strain produces many nodules it effects little or no fixation with any of the host varieties used here, a conclusion which is also to be drawn from the observations of Ruf & Sarles (1937) on this same strain in the Madison greenhouses. The decrease in final nitrogen content of the plants of Manchu (1939) and Black O varieties as compared with the original seeds is no doubt due to the negligible fixation, coupled with the fact that in the 1939 experiment the falling cotyledons were discarded instead of being preserved for addition to the plant material at harvest. Also there is the tendency to slight loss of nitrogen detected for similar plants in other experiments (see above). In the 1938 experiment the plants of two pots of Manchu beans inoculated with strain 507 grew vigorously and showed good fixation (see Table 1); it is certain that through some isolated error of technique the plants of these two pots became cross-infected with one of the more efficient strains, and no value is placed on the figures for the pots in question. The third pot was satisfactory. The fact that apart from the above instance good agreement was obtained between replicate pots, coupled with the existence in most cases of well-marked differences between the various combinations in respect of amount of nitrogen fixed, is indication that there was a general absence of cross-infection. For the same reasons statistical treatment of the data is unnecessary.

TABLE 1. *Amounts of nitrogen fixed by strains of the nodule organism when associated with different varieties of soya bean**

Bacterial strain	Pot no.	Manchu (1938)	Manchu (1939)	Brown C (1938)	Green Jap (1939)	Black O (1939)
505	1	264·5	202·4	169·8	131·9	192·2
	2	237·2	230·2	181·9	158·0	231·4
	3	232·6	189·7	136·0	110·0	244·4
	Means	**244·8**	**207·4**	**162·6**	**133·3**	**222·7**
9	1	153·6	182·8	125·3	151·9	245·3
	2	190·9	169·4	125·4	146·3	211·7
	3	182·6	169·8	94·5	107·9	265·8
	Means	**175·7**	**174·0**	**115·1**	**135·4**	**240·9**
17	1	46·9	61·5	69·1	125·4	157·4
	2	37·5	70·5	61·0	107·0	154·8
	3	31·2	53·2	49·5	84·9	164·5
	Means	**38·5**	**61·7**	**59·9**	**105·8**	**158·9**
507	1	198·0†	−1·4	−0·2	7·4	−9·0
	2	170·3†	−7·4	3·8	2·5	−8·2
	3	−0·7	−3·1	1·0	2·7	−8·5
	Means	**−0·7**	**−4·0**	**1·5**	**4·2**	**−8·6**

* In this table fixation is expressed as mg. N per pot of four (1938) or five (1939) plants. See text for method of computing fixation. The average N content of the original seeds in mg. per seed was as follows: Manchu (1938), 9·5; Manchu (1939), 9·1; Brown C, 9·6; Black O, 12·1; Green Jap, 13·8.

† Cross-infected, see text.

DISCUSSION

It has now to be considered whether the above results provide any evidence that the effectiveness of given strains of the nodule organism in contributing to the nutrition of the host plant is liable to vary when they are associated with different varieties of the host. Figures for fixation on a mg./plant basis are presented in Table 2. Certain conclusions as to changes in effectiveness could be drawn from an examination of these absolute figures

alone. Comparison will, however, be facilitated if we have in addition a relative expression of the figures. This is especially desirable since there were considerable differences of growth habit and nitrogen requirements between the different host varieties. There is also a need for a relative expression of fixation in dealing with observations made on the same combination of bacterial strain and host variety at different times and under somewhat different conditions, where variation in the absolute magnitude of fixation may again be expected as a result of the effect of environmental factors on the general growth of the plant, and thus indirectly on the activities of the associated bacteria. It is possible that in future experiments of this type a useful arrangement would be to grow, under the same conditions as the inoculated series, additional uninoculated plants of each variety, supplied with optimal amounts of combined nitrogen. The growth and final nitrogen content of these plants would serve as a standard against which the effectiveness of the various strains in supplying their hosts with fixed nitrogen could be assessed. In the present paper the somewhat less satisfactory method has been adopted of taking the nitrogen fixed by strain 505 as a basis for assessing the effectiveness of the three other strains. The appearance of the plants infected with this strain suggested that, although it was not always the best strain, it was of roughly comparable effectiveness with all four varieties, producing plants of a corresponding level of nutrition. The actual procedure has been to express the fixation by the three other strains with a particular host variety relative to that by strain 505 with the same variety, taking this as 100. These relative figures are also given in Table 2 and will be considered along with those for absolute fixation. It is appreciated that in so far as strain 505 did not maintain a constant level of effectiveness these relative figures may be misleading, e.g. an apparent increase in the effectiveness of another strain may actually be due to a decreased effectiveness of strain 505 itself.

TABLE 2. *Fixation of nitrogen expressed on an absolute (per plant) basis and also on a relative basis*

	Manchu (1938)		Manchu (1939)		Brown C (1938)		Black O (1939)		Green Jap (1939)	
Bacterial strain	Per plant*	Relative†	Per plant	Relative	Per plant	Relative	Per plant	Relative	Per plant	Relative
505	61·2	100	41·5	100	40·7	100	44·5	100	26·7	100
9	43·9	72	34·8	84	28·8	71	48·2	108	27·1	102
17	9·6	16	12·3	30	15·0	37	31·8	72	21·2	79
507	−0·2	0	−0·8	0	0·4	1	−1·7	0	0·8	4

* Fixation in mg. N/plant, derived from the mean values in Table 1.

† The figures in this and other corresponding columns indicate the fixation by strains 9, 17 and 507 with a given variety of host relative to that by strain 505 with the same variety, taking the latter as 100. See text.

The figures for the two experiments with the Manchu variety show that the order of decreasing effectiveness on each occasion was 505, 9, 17, 507, a finding which agrees with the observations of Ruf & Sarles (1937) in the Madison greenhouses, in which the same strains of organism (except 17) and variety of host plant were used. The fixation per plant by strains 505 and 9 was lower in 1939 than in the previous year. This is not, however, interpreted as signifying any actual deterioration in the effectiveness of the bacteria, but rather as being due to a limitation imposed on the growth of the plants, and indirectly on the activities of the associated bacteria, by a slightly closer planting and a less favourable

season. In the case of strain 17, however, the absolute fixation was higher in the second year, resulting in the relative figure being almost doubled, an observation which seems to suggest that the relative effectiveness of a given strain on a particular variety of host is not fixed, but may vary in different environments (see also Wilson *et al.* 1937; Raju, 1938). Proper attention should be paid to this possibility in considering the present results, and figures obtained for different varieties in different years should not be subject to direct comparison.

The variety Brown C is a smaller growing type than Manchu, and doubtless for this reason the absolute amounts of nitrogen fixed by strains 505 and 9 were lower than with the latter variety in the same year. Relative to each other these two strains were of almost precisely similar effectiveness with the two varieties. There was, however, a definite improvement in the effectiveness of strain 17 with Brown C, both on the absolute and relative basis, as compared with Manchu (1938). Strain 507 was again very ineffective.

With Black O, which was of similar growth habit to Manchu under the conditions employed, more marked changes in effectiveness appeared. The figures indicate that strain 9 now became of the same order of effectiveness as 505, while there was a further marked improvement in strain 17. Similar observations apply to the results for Green Jap, though this is a smaller-growing variety and the absolute fixation was considerably reduced compared with Black O. With both Black O and Green Jap, strain 507 continued extremely ineffective.

It is evident that like the field tests of Wright (1925), Erdman & Wilkins (1928), and Briscoe & Andrews (1938), the present, somewhat more controlled experiments indicate that the effectiveness of given strains of the soya-bean nodule organism may vary when they are associated with different varieties of the host plant. The variation revealed in these experiments is not, however, of a very drastic kind, and is chiefly in respect of one strain (17). This strain was relatively ineffective with the Manchu variety of host, but was effective with the Black O and Green Jap varieties. On the basis of the conclusions reached by Chen *et al.* (1940) it seems possible that this variation in effectiveness is due to varietal differences in the nature or amount of the substances affecting bacterial growth that are held to arise in the tissues of the host plant following on infection. Incidentally, in reference to the experiments of Chen & Thornton (1940), it may be noted that while in their case the nitrogen fixed per plant of soya bean inoculated with an efficient strain of organism, after 4 months' growth, amounted to 15 mg., in the present authors' experiments, where different strains were used, the fixation was considerably greater, and with one combination amounted to 61 mg. per plant. It would be of interest to ascertain whether in such cases the fixation, relative to the amount and duration of the bacterial tissue, is still of the same order as in the case of an ineffective strain such as 507.

From a practical point of view strains (such as 505 and 9) of the nodule organism which have been found to be the most effective fixers of nitrogen with American varieties of soya bean are likewise effective in conjunction with varieties which have been developed in an effort to meet the climatic conditions of growth in Great Britain. Dr H. G. Thornton informs us that his own tests at Rothamsted and Woburn lead to a similar conclusion.

Summary

Observations are presented on the fixation of nitrogen by four strains of the soya-bean nodule organism when associated with four varieties of the host plant, namely, Manchu, Brown C, Black O, and Green Jap. The plants were grown in sand culture in the greenhouse. The results agree with those of some previous investigators in showing that the relative effectiveness of some of the strains varied when they were associated with different varieties of host plant. The reason for the variation is discussed briefly. Consideration is given to the best basis for comparing the effectiveness of bacterial strains associated with host varieties of different growth habit, or with the same host variety under different conditions. Those strains which were effective with the American variety (Manchu) were also effective with the British varieties.

The authors acknowledge a contribution from the Carnegie Trustees towards the cost of publication.

REFERENCES

Bond, G. (1936). Quantitative observations on the fixation and transfer of nitrogen in the Soya bean, with especial reference to the mechanism of transfer of fixed nitrogen from bacillus to host. *Ann. Bot., Lond.*, **50**, 559.

—— (1938). Excretion of nitrogenous substances from leguminous root nodules: observations on Soya bean. *Ann. Bot., Lond.*, N.S., **2**, 61.

Briscoe, C. F. & Andrews, W. B. (1938). Effect of strains of nodule bacteria and lime on the response of soybeans to artificial inoculation. *J. Amer. Soc. Agron.* **30**, 711.

Chen, H. K. & Thornton, H. G. (1940). The structure of 'ineffective' nodules and its influence on nitrogen fixation. *Proc. roy. Soc.* B, **129**, 208.

Chen, H. K., Nicol, H. & Thornton, H. G. (1940). The growth of nodule bacteria in the expressed juices from legume roots bearing effective and ineffective nodules. *Proc. roy. Soc.* B, **129**, 475.

Erdman, L. W. & Wilkins, F. S. (1928). Soybean inoculation studies. *Bull. Ia. agric. Exp. Sta.* no. 114, p. 1.

Fred, E. B., Baldwin, I. L. & McCoy, E. (1932). *Root nodule bacteria and leguminous plants.* Madison, Wis.

Raju, M. S. (1938). Studies on the bacterial-plant groups of cowpea and Cicer. V. Symbiotic fixation of nitrogen. *Zbl. Bakt.* 2 Abt. **99**, 289.

Ruf, E. W. & Sarles, W. B. (1937). Nodulation of soybeans in pot culture by effective and ineffective strains of *Rhizobium japonicum*. *J. Amer. Soc. Agron.* **29**, 724.

Strong, T. H. (1940). Non-effective associations of nodule bacteria and legumes, with particular reference to the influence of the host plant. *J. Aust. Inst. agric. Sci.* **6**, 14.

Wilson, P. W. (1940). *The biochemistry of symbiotic nitrogen fixation.* Madison, Wis.

Wilson, P. W., Burton, J. C. & Bond, V. S. (1937). Effect of species of host plant on nitrogen fixation in Melilotus. *J. agric. Res.* **55**, 619.

Wright, W. H. (1925). The nodule bacteria of soybeans. II. Nitrogen fixation experiments. *Soil Sci.* **20**, 131.

(*Received* 21 *November* 1941)

INVESTIGATIONS ON POTATO ROOT EELWORM, *HETERODERA ROSTOCHIENSIS* WOLLENWEBER

ON THE CYST POPULATION OF A FIELD OVER A SERIES OF YEARS

By HERBERT W. MILES, D.Sc., Ph.D. and MARY MILES, M.Sc.
University of Manchester

In 1927 and 1928 potato sickness was widespread and severe in potato-growing areas of Lancashire and Cheshire, and losses were heaviest where potatoes were grown at short intervals. The potato-root eelworm, *Heterodera rostochiensis* Wollenweber (*H. schachtii* Schmidt) (Franklin, 1940), was present in the soil of all potato-sick fields examined, and although Morgan (1925, 1926) had shown that there was no direct association between cyst population of the soil and intensity of the pathological condition known as potato sickness, the eelworm infestation of the soil was considered an important factor in the incidence of the disease.

In the absence of suitable direct measures against potato sickness it had been suggested that widening the rotation, so that potatoes were grown at longer intervals, might reduce loss. The failure of potatoes on certain fields at the Potato Testing Station at Ormskirk in 1928 caused the Superintendent to modify the cropping system usually followed and temporarily to increase the interval between potato crops. On one field where the crop failed in 1928 potatoes were not grown again until 1936. With the co-operation of the Superintendent, part of this field, which was known to be infested with potato-root eelworm, was kept under observation. The study of the cyst population began in June 1929 and is still in progress.

Scope of the observations

Little is known of the duration of life of cysts of the potato-root eelworm and of changes in the cyst population through the absence of the host plant. In his study of the beet eelworm, Thorne (1923) indicated that there was apparently a decrease in the number of eggs remaining in the cysts corresponding to the number of years the land had not been planted with sugar beet, but studies of fields on which beets were not cultivated for periods of 1–12 years showed that there was little or no possibility of eradicating the eelworm from the soil by methods of rotation applicable to general farm practice. It was thought that observations on the cysts of potato-root eelworm during a period in which potatoes were not cultivated would give some information concerning the time the cysts remained viable in soil under natural conditions. The effect of resting the land from potatoes on the incidence of potato sickness in subsequent crops was also observed.

Method of measuring cyst population

The area under observation, approximately $\frac{1}{2}$ acre in extent, was divided into nine plots in order to ensure that each set of observations was equally well distributed over the area. A general sample, consisting of a number of random borings with an auger, was taken from each plot in June and again in December of each year. The soil was air dried and passed through a 2 mm. sieve, and ten samples each of 10 c.c. were examined from each general sample. The flotation method was used to isolate the cysts.

From June 1929 to December 1935 the cysts were counted on the filter papers and only 100–200 were removed to microscope slides for examination for viability. From June 1936 all cysts were removed from the filter papers to microscope slides for further examination, and records were kept of their character and viability. Cysts were recorded as viable when they contained larvae or viable eggs. No test of viability was applied to the eggs, but eggs that were normally translucent white under the microscope were considered viable. Empty eggs were readily distinguished, and discoloured eggs were considered not viable.

The more critical examination of the cysts that was part of the technique after December 1935 revealed that among the potato-root eelworm cysts were some small delicate, lemon-shaped cysts of another species of *Heterodera*. These cysts were excluded from the cyst population counts from June 1936 onwards.

Error of the method. In a paper by Smith & Prentice (1929) the percentage standard error of the laboratory and field technique of this method of making observations on the cyst population is discussed. The soil of the field on which these observations were made was a peaty sand, and Smith & Prentice estimate that for peaty sands the standard error associated with laboratory technique is about 7%, that associated with field technique about 13%, and the total error of the method about 14·7%.

The standard error of the laboratory technique has been calculated for ten series of the observations summarized in Table 1. The average standard error for each series is as follows:

	%		%
June 1929	7·7	June 1936	10·4
December 1930	8·0	December 1937	9·8
„ 1931	8·2	„ 1938	9·9
June 1933	7·1	June 1939	9·3
„ 1934	9·1	December 1939	8·2

These percentages are generally higher than those of Smith & Prentice, the reason apparently being that the cyst population of the area under observation was lower than that on which these authors based their calculations. Smith & Prentice arbitrarily excluded counts of less than ten cysts per 10 c.c. of soil, since the effect of samples with a very small number of cysts was to increase the standard error abnormally. In this investigation populations of less than ten cysts per 10 c.c. of soil were frequent in every series of counts and in some of the series populations of more than ten cysts per 10 c.c. of soil were rare.

Some variation in the numbers of cysts from point to point on a plot is to be expected. An estimate of the error involved in field sampling can be made from a comparison of ten general samples from plots B and G in December 1940. Counts of cysts were made in the usual way and the following figures obtained:

Sample	Mean of 10 counts	Sample	Mean of 10 counts
B 1	7·1	G 1	7·8
B 2	6·5	G 2	5·9
B 3	9·0	G 3	7·0
B 4	5·1	G 4	8·2
B 5	7·0	G 5	9·1
B 6	6·8	G 6	7·0
B 7	5·1	G 7	7·6
B 8	5·3	G 8	7·7
B 9	6·2	G 9	7·3
B 10	5·0	G 10	6·6
Standard error	6·3%	Standard error	3·8%

It has not been possible to calculate a total standard error as did Smith & Prentice, since the conditions under which the observations were made rendered uniformity in field sampling difficult. In some years the soil was so dry in June that it could not be withdrawn by the auger, consequently the samples were taken with a trowel. When the field carried a cereal crop random sampling could not be done without injuring the crop, and in these circumstances the samples were taken in an irregular line along the drills near the middle of each plot. At the beginning of the observations twenty-four borings composed each field sample, but in the course of time sampling became less accurate and the field samples came to consist of 20–24 borings or soil taken from the tip of the trowel at 20–24 points on the plot.

Cultivation and cropping during the period of observation

The usual rotation for the district where the observations were made is a 3-year cycle consisting of cereals, hay, and potatoes. In 1928 the field under observation was cropped with potatoes and the yield obtained was less than 2 tons/acre. To rest the field from potatoes the rotation was modified and extended by leaving the ley for 2 years and substituting cabbage for potatoes in the fourth year. In the eighth year potatoes were grown, and subsequently the usual 3-year rotation was followed. Cultivation was that customary for the crops grown. In the autumn of 1935 the land was broken with a gyrotiller in preparation for the 1936 crop. This operation seemed to increase the numbers of cysts in the samples but it had no noticeable influence on the effective cyst concentration (Table 1). The potato crop of 1936 was healthy and vigorous. The haulms were strong and even,

Table 1. *Number of cysts per* 100 *c.c.* 1929–40

Date	Crop	A	B	C	D	E	F	G	H	I	Total	Average per 10 c.c.	Viable cysts %	Effective cyst conc. per 10 c.c.
June 1929	Wheat	153	129	138	216	121	133	183	122	117	1312	14·6	87·1	12·7
Dec. ,,		91	107	115	170	79	101	119	117	113	1012	11·2	77·9	8·7
June 1930	Hay	106	73	78	104	60	78	101	87	87	774	8·6	71·7	5·7
Dec. ,,		115	109	93	142	91	107	158	81	102	998	11·1	62·9	7·0
June 1931	Hay	73	95	66	125	43	67	84	64	101	718	8·0	56·8	4·6
Dec. ,,		115	108	103	143	115	105	95	100	91	975	10·8	48·0	5·2
June 1932	Cabbage	103	99	118	125	77	82	97	93	98	892	9·9	45·8	4·5
Dec. ,,		125	94	112	114	123	116	114	86	131	1015	11·2	39·3	4·4
June 1933	Oats	71	116	107	143	94	110	114	98	103	956	10·6	33·6	3·6
Dec. ,,		128	114	141	137	115	97	111	135	126	1104	12·3	32·2	4·0
June 1934	Hay	90	79	70	85	77	70	82	59	92	704	7·8	35·5	2·8
Dec. ,,		64	61	83	76	51	76	51	82	74	618	6·9	34·5	2·4
June 1935	Hay	104	102	97	109	66	71	81	81	79	790	8·8	—	—
Dec. ,,		167	118	151	125	86	103	141	137	88	1116	12·4	21·9	2·7
June 1936	Potatoes	84	74	93	102	41	80	108	83	73	738	8·2	1·8	0·14
Dec. ,,		133	114	81	117	108	105	93	118	137	1006	11·2	29·3	3·3
June 1937	Wheat	138	75	105	85	36	67	86	60	107	759	8·4	23·6	2·0
Dec. ,,		107	88	84	67	61	87	78	72	99	743	8·3	25·7	2·1
June 1938	Hay	79	44	85	80	97	83	77	60	73	678	7·5	27·0	2·0
Dec. ,,		89	58	96	69	63	88	96	52	98	709	7·8	23·7	1·9
June 1939	Potatoes	95	70	68	68	84	46	61	69	74	635	7·1	14·6	1·0
Dec. ,,		198	99	189	100	135	127	111	173	216	1348	15·0	62·7	9·4
June 1940	Wheat	154	57	83	131	82	87	89	160	131	974	10·8	26·3	2·8
Dec. ,,		94	71	68	78	56	113	78	60	105	723	8·0	16·5	1·3

and the roots formed a large fibrous mass. The yield was well above the average for the district. The 1939 potato crop was also healthy and showed none of the signs associated with potato-root eelworm disease. The yield was satisfactory.

TABLE 2. *Number of viable spherical cysts* 1936–40

Date	Crop	Viable cysts per 100 c.c. soil										Average per 10 c.c.
		A	B	C	D	E	F	G	H	I	Total	
June 1936	Potatoes	0	0	2	1	1	2	4	1	2	13	0·14
Dec. ,,		28	38	26	33	38	27	21	30	54	295	3·3
June 1937	Wheat	48	17	34	7	3	17	0	8	45	179	2·0
Dec. ,,		31	21	17	17	23	21	18	15	28	191	2·1
June 1938	Hay	24	6	25	19	33	17	21	13	25	183	2·0
Dec. ,,		20	8	25	18	15	22	15	9	36	168	1·9
June 1939	Potatoes	17	5	3	8	21	10	9	11	9	93	1·0
Dec. ,,		123	51	134	64	81	89	60	112	131	845	9·4
June 1940	Wheat	50	8	7	41	17	27	14	62	30	256	2·8
Dec. ,,		18	11	9	13	9	22	14	7	16	119	1·3

CHANGES IN CYST POPULATION

A summary of data collected from June 1929 to December 1940 is given in Table 1. The two parts of the table should be considered separately because of differences in technique already described, and because numbers in the first part of the table refer to total cyst population, while those in the second refer to cysts of the potato-infesting species only.

Table 1 shows that in the absence of the host plant the percentage of viable cysts and the effective cyst concentration declined. The period of 7 years between potato crops was not long enough for all cysts to lose their viability. The potato crops of 1936 and 1939 were associated with increases in the percentage of viable cysts and in the effective cyst concentration.

It will be noted that the original cyst population of the soil was not high, although the failure of the crop in 1928 was associated with eelworm attack. The persistence of cysts in the soil is demonstrated. In June 1936, before new cysts were liberated into the soil from the 1936 potato crop, over 50% of the original cyst population remained in the soil in spite of the disintegrating effects of weather and cultivation.

In data collected up to December 1935 there is some evidence of new cysts being added to the soil, since cyst counts for December are frequently higher than those for June of the same year. A re-examination of such early samples of soil as were available showed that small, delicate lemon-shaped cysts of another *Heterodera* species were present in varying numbers. These may have been associated with crop plants or with weeds, and their presence may explain the increase in cyst population that occurred in December of some years.

Table 2 and the latter part of Table 1 give data concerning the potato-infesting species of *Heterodera* only. The effects of the presence of the host plant on the cyst population are clear. Hatching and emergence of larvae from cysts were stimulated so that percentages of viable cysts in June of the years potatoes were grown showed a marked reduction over those of the previous December. Soil samples taken after the crop was lifted showed that the cyst population was greatly increased by the cultivation of the host plant.

No satisfactory explanation of the drop in numbers of viable cysts and in effective cyst

concentration in the year following a potato crop can be given, but the following suggestions are made. The presence of residual plants from previous potato crops would offer some stimulus to hatching. Traces of the stimulating substances may remain in the soil during the winter after the potato crop and have some effect on the cysts in the following spring. The contents of small, delicate flask-shaped cysts that did not complete their development before the crop matured may have dried during the cultural operations carried out in the winter and spring.

Condition of cysts in 1936

In June 1936, practically 8 years after potatoes had been grown on the field, the number of unbroken spherical cysts was approximately 7 per 10 c.c. of soil. Most of the cysts were empty, but in some there were empty egg shells. Of 238 unbroken cysts from soil taken in June 1936 only three were viable. One of these contained sixty-four eggs that appeared viable, twelve eelworm larvae and a number of empty egg shells; another contained egg shells, five larvae and four viable eggs; and the third contained six larvae and some egg shells. The cysts from soil samples taken in June 1936 had been exposed to the effects of the early growth of the 1936 potato crop.

Cyst population and the potato crop

From these observations it is apparent that potato-root eelworm is not the only factor concerned in potato sickness (Miles, 1930; Buckhurst & Fryer, 1931). In 1939 the cyst population was similar to that obtaining in 1929. In 1928 the potato crop was a failure; in 1939 the crop was entirely satisfactory. This is further evidence that in the field factors other than potato-root eelworm play a decisive part in the incidence of potato sickness. The prolonged rest from potato crops enabled these factors to become so adjusted that normal, healthy potato plants were produced in the presence of potato-root eelworm infestation.

Summary

In the absence of the host plant there is a gradual decrease in the viability of cysts of the potato-root eelworm. The period of 7 years between the potato crops of 1928 and 1936 was not long enough for all cysts to lose their viability. Two crops of potatoes with a 3-year interval were sufficient to restore the cyst population to the level obtaining before the period of 7 years without a potato crop. The interval of 7 years between potato crops was associated with the disappearance of all signs of 'potato sickness' in the two subsequent crops.

The writers thank Mr J. C. F. Fryer, M.A., Chief Entomologist to the Ministry of Agriculture, for his continued and helpful interest in field work on the potato-root eelworm, and also Dr F. Yates, M.A., and Mr D. Finney, M.A., of the Rothamsted Experimental Station for reading the paper and making useful criticisms.

REFERENCES

Buckhurst, A. S. & Fryer, J. C. F. (1931). The problem of 'potato sickness'. *Ann. appl. Biol.* **18**, 584.

Franklin, M. T. (1940). On the specific status of the so-called biological strains of *Heterodera schachtii* Schmidt. *J. Helminth.* **18**, 193.

Miles, H. W. (1930). Field studies on *Heterodera schachtii* Schmidt in relation to the pathological condition known as 'potato sickness'. *J. Helminth.* **8**, 103.

Morgan, D. O. (1925). Investigations on eelworm in potatoes in south Lincolnshire. *J. Helminth.* **3**, 185.

—— (1926). Some remarks on the etiology of potato disease in Lincolnshire. *J. Helminth.* **4**, 49.

Thorne, G. (1923). Length of the dormancy period of the sugar-beet nematode in Utah. *Dep. Circ. U.S. Dep. Agric.* no. 262.

Smith, A. M. & Prentice, E. G. (1929). Investigations on *Heterodera schachtii* in Lancashire and Cheshire. *Ann. appl. Biol.* **16**, 324.

(*Received* 30 *November* 1941)

OBSERVATIONS ON THE BIOLOGY OF THE CARROT FLY (*PSILA ROSAE* FAB.): ASSEMBLING AND OVIPOSITION

By F. T. BAKER, F.R.E.S., I. E. KETTERINGHAM, N.D.A., S. P. V. BRAY, B.Sc. and J. H. WHITE, B.Sc.
Midland Agricultural College

(With 6 Text-figures)

Observations of Mr A. Roebuck, Advisory Entomologist in the Midland Agricultural Advisory Province, over many years tended to show that the emergence and oviposition of the spring generation of the carrot fly was an abrupt phenomenon. On the other hand, the emergence of the second generation in the late summer, and possible partial third generation in the autumn in some years, was prolonged. The prospects of controlling the pest by destruction of the adults, either before or immediately after emergence, would be greater, therefore, in the spring than at any other time. Puparia were collected in the fields in March and kept under warm conditions in the laboratory: adults emerged in the middle of May. Experience of previous years was that indoor emergence usually preceded outdoor emergence by about 10–14 days, and it was thought therefore that the peak emergence in north Lincolnshire would be the end of May or beginning of June. At Mr Roebuck's suggestion the writers undertook to study the flies in the field as intensively as possible until the completion of the egg-laying period. For the most part the writers worked in pairs, each pair being relieved every few days. The observations reported cover the period 6 June–2 July 1941.

In order to eliminate certain complications it was decided to study the problem in a recently developed carrot-growing area, rather than in a district where large acreages of carrots had been grown over a number of years. Kirmington, on the wolds in north Lincolnshire, was selected, as it was known that considerable infestation of carrot fly occurred there in 1940. Two farmers had grown carrots since 1930 and another had a crop for the first time in 1940. They were grown successfully for several years, and the crops were at first quite free from carrot-fly damage. Infestation appeared on the two farms at about the same time, though the precise date of its first appearance was not known. Each year following the first attack, infestation occurred in varying degree. In 1940, the clamped carrots were affected, the attack being fairly severe.

Through the courtesy of Mr S. Fisk, facilities for study were granted on the Manor Farm, Kirmington, where 15 acres of carrots were grown in 1940 and 20 acres in 1941. The fields were close together but not adjoining (see Fig. 1); it was hoped that this would give opportunity to study movement or migration from the place of emergence (i.e. the site of last year's carrot crop) to the current year's crop. The fields were enclosed by hawthorn (*Crataegus Oxyacantha* L.), hedges varying from 2 to 10 ft. in height; trees and other bushes occurred in places. Growth in the hedge bottom varied but chervil (*Chaerophyllum sylvestre* Linn.) and hogweed (*Heracleum Sphondylium* L.) were abundant (see Fig. 1). The soil was well-drained, light, chalky loam.

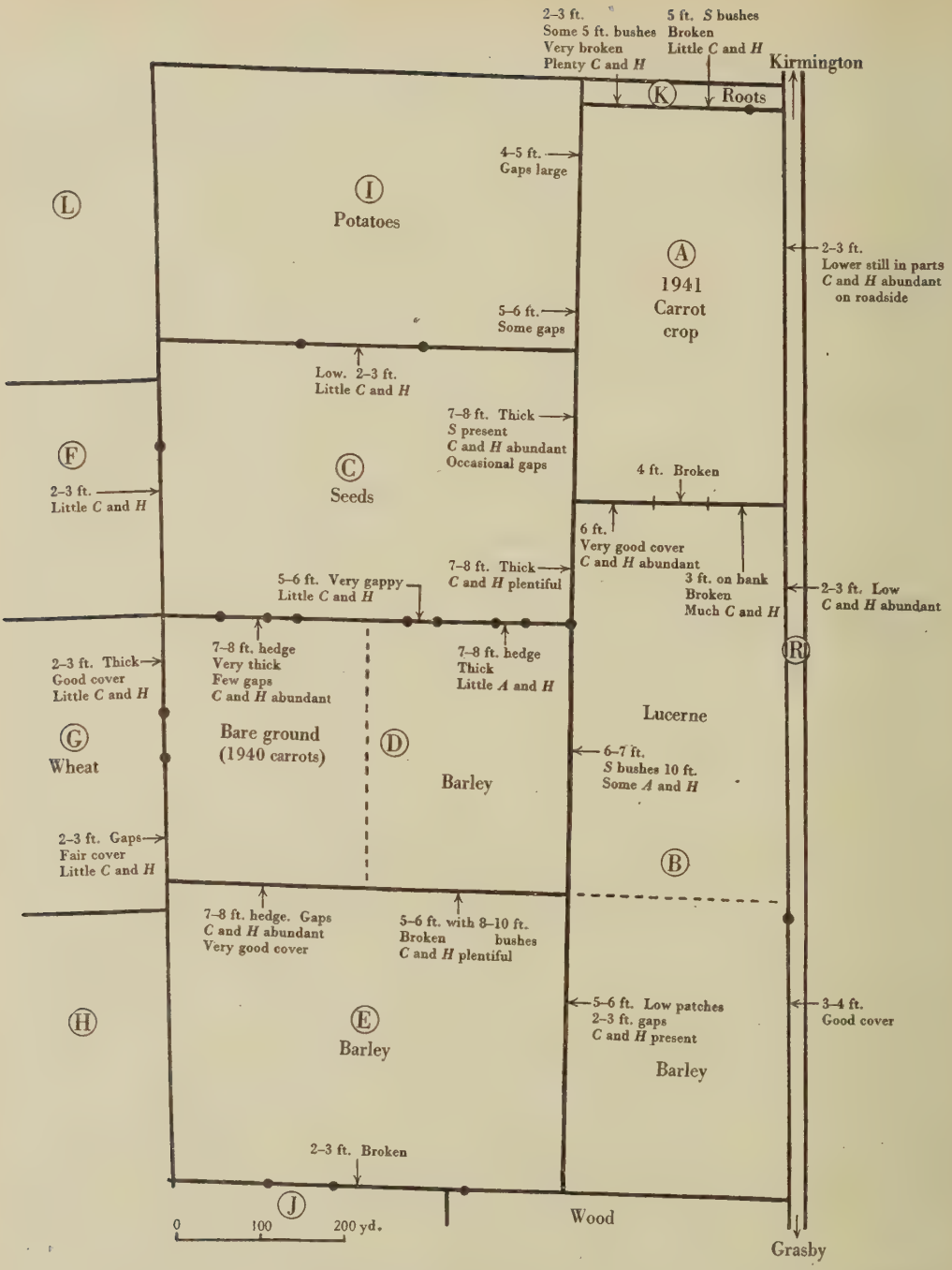

Fig. 1. Field plan showing types of hedges and undergrowth in the vicinity of 1940 and 1941 carrot fields. *C* = *Chaerophyllum sylvestre* L. *H* = *Heracleum Sphondylium*. *S* = *Sambucus nigra* L.

Note. Hedges are always referred to in the text by the field letters separated by the hedge. E.g. Hedge between fields A and B is AB.

Technique

The investigation was a preliminary survey, and in the light of new observations the technique was varied as the work progressed. On the first visits very few flies were observed out in the fields, and the flowers of chervil and hogweed by the roadside were swept without result. Traps of molasses also gave negative results. Therefore, it was considered desirable to examine the 1940 crop field as being the source of the 1941 brood. In this field the flies were present in small numbers on the chervil and hogweed, but subsequent search revealed their numbers to be considerable in the hawthorn hedges. This suggested lines for further work and the following technique was evolved.

Sweeping. Using an entomological sweep-net, trial sweeps on the hedges of the 1940 carrot field were made to determine the best method of obtaining indications of the distribution and concentration of the fly. Hedges were swept on both sides and the counts indicated heavy concentrations on the sheltered side and few on the windward side, although the breeze was very slight. Continual checks confirmed this preliminary finding. On one occasion only, in the complete absence of wind, the method did not give an accurate comparison as the flies were distributed evenly throughout the hedge. Uniformity in the method of sweeping was obtained by taking one sweep for each pace forward along the hedge, taking twenty-five sweeps in all; the carrot flies in the net were counted at the end of the 25th sweep—this was designated the *standard sweep*. Standard sweeps were taken at intervals along the hedge. All sections of the hedge, high, medium and low, were covered during each standard sweep.

Traps. In order to determine the movement of flies in the field, traps were constructed. These were wood blocks 2 × 2 × 1 in. covered with a sticky preparation. Various compounds were tried including molasses, fly-papers and 'Stictite' grease-banding; the latter was found to be most satisfactory, retaining its stickiness under all conditions. In the first place traps were placed in pairs, one on the ground and the other 1 ft. above the ground. The latter did not catch any carrot flies and therefore were replaced by additional ground traps. One of each pair was then placed in the drill-row, the other between the drill-rows in close proximity. At the first sign of loss of efficiency the sticky papers were renewed. The traps were distributed about the field (see Fig. 2) with the

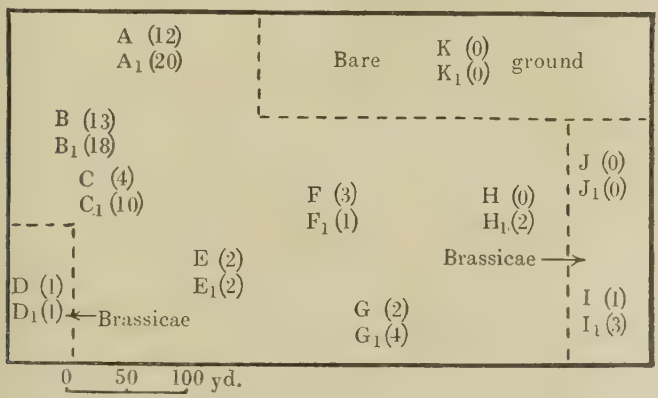

Fig. 2. Position of traps in 1941 carrot field. (Figures in brackets indicate number of flies caught on each trap.)

following objects: (*a*) to give an indication of the numbers of flies in the field, (*b*) to show distribution of flies in the field, (*c*) to show affinity, if any, for carrot seedlings, (*d*) to indicate periods of oviposition, and (*e*) to determine area of oviposition. For the purpose of chemotropic experiments these traps were drilled with 1 in. holes in which a wad of cotton-wool was inserted soaked with the appropriate chemical; the paper was perforated and fixed over the block as before.

Direct observations. These were also made at various times with a view to obtaining facts relating to the habits and biology of the fly, particularly with regard to oviposition.

Results

Distribution

Assembly of flies in the hedges. The tendency of carrot flies to assemble in large numbers in the hawthorn hedges was very marked. This was particularly so in the case of the males, only one male being taken on the traps in the field, as against 170 females, during the whole of the period of these observations. This habit of assembling appears to be affected by time of day and climatic conditions. Traps in the field indicated that there was no marked movement from the hedge until approximately 4 p.m. (G.M.T.), and that the majority of the flies returned to the hedge at nightfall (see Fig. 6). In dull, cool, windy weather little movement takes place from the hedge (see Fig. 4: 19 June). During a prolonged spell of warm weather with little wind, at least half the flies were out in the field during the period of movement. This was inferred from the fact that during the heat wave from 20 to 26 June relatively few flies were caught in the hedges and the population remained constant (see Fig. 5: 20–26 June), but when the heat wave broke, the flies were once more to be found in greater numbers in the hedges (see Fig. 5: 27–28 June).

Abundant growth of chervil and hogweed alone did not attract large numbers of the fly. It was only when additional cover was afforded by a hedge that sweeps showed appreciable numbers on these plants, and even then the proportion in the hedges was always higher. This is shown by the following figures:

Date June	Hedge	Height ft.	No. of *P. rosae* per standard sweep on chervil	No. of *P. rosae* per standard sweep in hedge
6	AR	2–3	0	0
12	AB	6	10	41
13	AB	6	5	10
13	CD	7–8	20	50

The low roadside hedge (AR) with prolific growth of chervil and hogweed on both sides consistently showed low counts. The average numbers of flies in standard sweeps in different hedges is summarized in Table 2.

Susceptibility to wind changes. Sweeping indicated that the majority of flies was always on the sheltered side of the hedge when the wind was blowing through it (see Table 1), even when the wind was of low velocity. The quick response of the flies to a change in wind direction was most marked. On 13 June at 3 p.m. (G.M.T.) there was a fresh breeze from north-west which quickly changed to south-south-east at approximately 6 p.m. At 6.10 p.m. when the hedge DC was swept the carrot flies had moved to the now sheltered side of the hedge, the standard sweeps yielding forty on the sheltered side and one on the exposed side. The effect of a drop in the wind was to cause an equal distribution throughout the hedge. For example, on 16 June at 6.30 p.m. there was a fresh breeze from south-west, and while sweeping the hedge DC the wind dropped. Sweeps on both sides of the west end of the hedge revealed a tendency to a more even distribution: five and four on one side, seven and fourteen on the other. The following evening at 6 p.m. approximately, on the same hedge with a strong south-west wind comparative sweeps gave twenty-five and fifteen on the sheltered side and none on the other.

Effect of gale on local movement. On 10 June with a wind of gale force, the carrot flies left the upper part of the hedge and assembled in the undergrowth on the sheltered side,

TABLE 1. *Evidence of the habit of the carrot fly of assembling on sheltered side of hedge*

Date	Time G.M.T.	Hedge (see Fig. 1)	Wind	Sweeps: No. of flies on sheltered side	Sweeps: No. of flies on exposed side
11 June	12 noon–1.00 p.m.	EH	N.	*7	6
13 June	5.00 p.m.	AR	S.S.E.	8	1
	5.30	AI	S.S.E.	8	1
	6.00	BC	S.S.E.	7	0
	6.10	DC	S.S.E.	40	1
	6.20	DG	S.S.E.	5	2
	7.30	DB	S.S.E.	5	4
16 June	5.00	AR	S.W.	10	4
	6.40	DC	S.W.	†7	5
	6.40	DC	S.W.	14	4
	6.40	DE	S.W.	12	4
	6.45	DB	S.W.	7	2
19 June	4.30	DC	E.	*0	0
	4.35	DC	E.	*1	1
	4.50	GF	E.	*0	1
	5.30	GH	E.	*0	0
	6.00	DE	E.	21	1
20 June	10.30 a.m.	AR	S.E.	3	0
	10.30	AR	S.E.	2	0
	11.30	AI	S.E.	‡1	2
	11.30	AI	S.E.	‡0	7
	12.00 noon	AC	S.E.	‡0	7
	12.30 p.m.	IL	S.E.	0	0
23 June	5.30	AB	S.W.	0	0
	6.00	DC	S.W.	‡0	3
	6.10	DC	S.W.	‡6	0
	6.20	DC	S.W.	‡1	0
26 June	5.30	AR	N.W.	0	0
	5.50	AB	N.W.	4	0
30 June	5.00	DC	S.S.E.	0	0
	5.10	DC	S.S.E.	4	0
	5.30	DG	S.S.E.	0	0
	5.35	DG	S.S.E.	0	1
	6.00	DB	S.S.E.	2	1
	6.10	CB	S.S.E.	2	0
	7.30	AC	S.S.E.	0	0
1 July	4.00	DE	N.E.	†4	0
	4.30	DC	N.E.	†0	0
	4.40	AB	N.E.	†2	0
	4.40	AB	N.E.	†0	2

* Wind along the hedge. † Sudden drop in wind.
‡ Sweeping inaccurate on sheltered side owing to sheep-netting.

composed largely of chervil and hogweed. The flies gathered on the undersides of the leaves of these plants—more particularly on hogweed as its leaves afforded better protection from the wind. They were lethargic and showed little inclination to move—four pairs were noted copulating under these conditions. With a strong wind blowing the flies ventured only short distances from the hedge. On 19 June with a strong wind from north-east the traps in both fields gave a small number of females out in the field (see Fig. 4).

Shelter in relation to concentration of flies. This factor appeared to be important. Dense, high hedges with abundant bottom growth definitely attract the fly and govern the distribution of high concentrations. High hedges at the source of emergence greatly reduced

the possibility of the flies being dispersed over a wide area. This point was illustrated by the fact that a considerable population in the 1940 carrot field, with many favourable

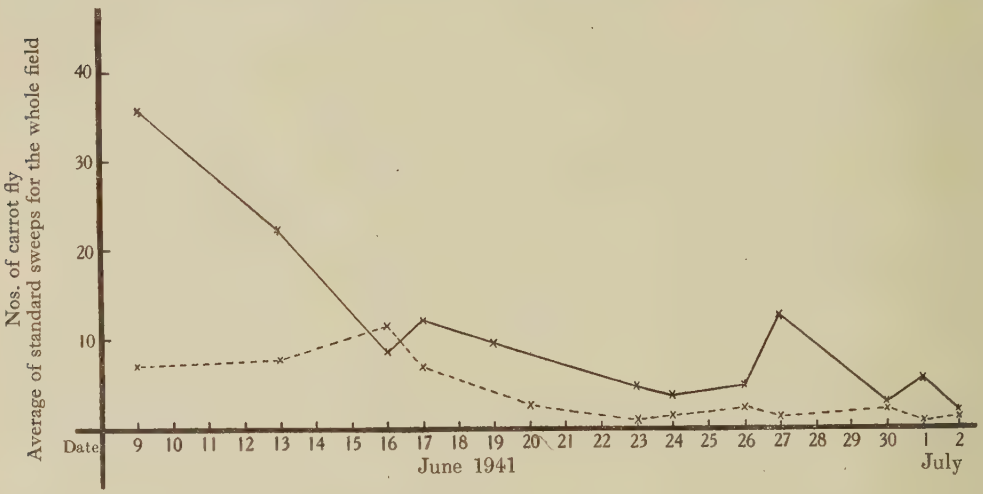
Nos. of carrot fly
Average of standard sweeps for the whole field
40
30
20
10
Date
9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 1 2
June 1941
July

Fig. 3. Variation in numbers of carrot fly in 1940 and 1941 fields. ——— 1940. – – – – – 1941.

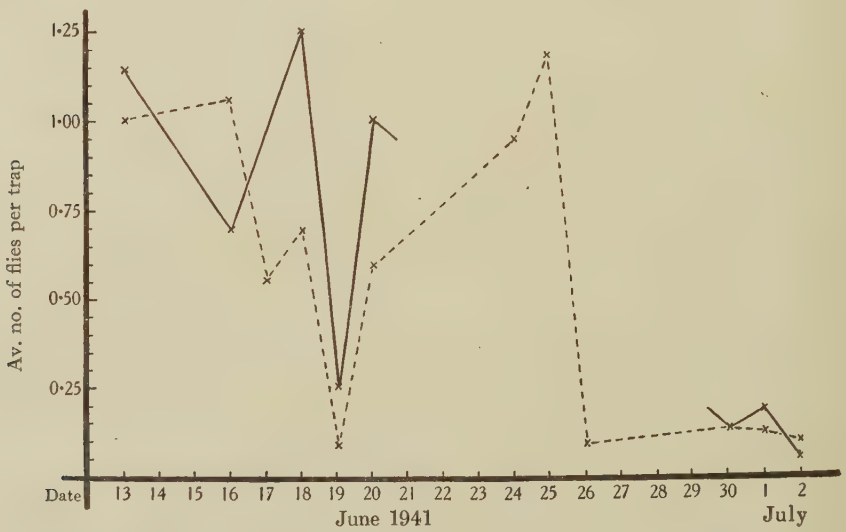

Fig. 4. Variation in numbers of carrot fly taken on traps in 1940 and 1941 fields.
Note. 20–30 June records interrupted in 1940 field owing to cultivation.

stretches of hedge, was maintained throughout the whole of the life period of the fly (see Fig. 3).

Wind dispersal. Results suggested that the movement of the carrot flies was in the nature

of a general dispersal rather than a well-defined migration. The reasons for this conclusion were as follows:

(1) *Evidence from* 1940 *crop field* (*D*). This field was chosen for special study as it was the source of the flies (emerging from the sites of clamps, etc., of the last year's carrot crop), and retained a large number of flies throughout the period of observation. The 'average sweeps' (i.e. average of the standard sweeps for a particular hedge) of the four hedges were plotted against the time and the results are given in Fig. 5.

TABLE 2. *Average numbers of flies in standard sweeps in different hedges*

Hedge (see Fig. 1)	June														July	
	6	9	11	12	13	16	17	19	20 10 a.m.	20 4 p.m.	23	24	26	27	30	1	2
AB	5	25	—	13·3	8	13·3	5·6	3·5	21·25	5	0·3	1·6	2·6	0·3	3·0	0·6	2·0
AR	0	0·3	—	—	4	8·75	6·3	—	1·75	2·5	0·6	0·3	1·0	0·3	1·3	0·6	0
AK	0	3·0	—	—	8	4·5	1·5	—	4·6	0·6	0·0	0·3	1·6	1·0	3·6	0·3	0·6
AI	—	5·0	—	—	11·5	11·5	14·5	—	2·5	1·3	1·0	0·5	3·0	3·5	0·0	0·5	2·0
AC	0	3	—	—	11	25	8	—	2·3	3	3	6	3	1	0	0	0
BC	15	2	—	—	4·5	6·5	25	0·5	4·0	—	25	11·5	6·5	8	1	0	1·5
DB	15	7	—	—	5·0	7·3	9·3	5	—	—	5·3	4·3	4	13	0·6	1	1
DC	50	60	—	5	22	10	12·75	2·3	—	—	4	1·75	3·5	10·5	2	0·25	1
DG	60	60	—	20	6	6·3	16	28	—	2·2	1·6	4·3	0·3	4·6	1	5·6	0
DE	50	30	—	38	56·6	9·6	9·6	7·5	—	21·5	7·25	4·75	8·6	19·25	6·5	15·3	3·4
CI	—	—	—	—	—	—	—	—	5·6	1·6	—	—	—	—	—	—	—
CF	—	—	—	—	—	—	—	—	2	1·5	—	—	—	—	—	—	—
EH	0	—	6·6	—	—	—	—	0	—	—	—	—	—	—	—	—	—
EJ	0·5	—	8·6	—	—	—	—	0·3	—	—	—	—	—	—	—	—	—
EB	0	—	—	—	—	—	—	5·6	—	—	—	—	—	—	—	—	—
GF	4·5	26·7	—	—	—	—	—	0·25	—	10·5	—	—	—	—	—	—	—
GN	17	20	—	—	—	—	—	4·0	—	—	—	—	—	—	—	—	—
GM	17	10	—	—	—	—	—	0	—	—	—	—	—	—	—	—	—
GH	12	13·6	1·5	—	—	—	—	1	—	—	—	—	—	—	—	—	—
HN	—	—	—	—	—	—	—	11	—	—	—	—	—	—	—	—	—

Between 9 and 12 June the numbers (as indicated by the 'average sweeps'—see Table 2) in the hedges DC and DG fell considerably more than could be explained by experimental error or the general decrease due to death. At the same time the numbers in DE rose slightly; those in DB remaining practically constant. This fluctuation coincided with prevailing winds from north-east and north. Thus, there had been a tendency for the flies to leave the windward hedge DC and accumulate in DE. During the period 20–26 June the graph indicates little movement of the flies from hedge to hedge; this coincided with a period of slight, variable breezes and a general heat wave.

(2) *Evidence from adjoining fields.* In the field E an 'average sweep' of 0·5 was obtained in the hedge EJ on 6 June. This had changed on 11 June to an average of 8·6, and during this period the wind had been consistently in the northerly quarter, probably carrying flies across the adjoining field E, from the hedge DE of known high concentration (see Table 2).

The average numbers of flies present in the hedges of the 1941 carrot field were maintained consistently at a lower level than in the source field (see Fig. 3). This would not be expected if there was a general migration to the nearest carrot crop. There is also a lack of any outstanding correlation between the 'rises' in the 1941 field and the 'falls' in the 1940 field, apart from the slight 'rise' in the 1941 field from 9 to 16 June which corresponded to the rapid 'fall' in the 1940 field; this was no doubt part of the general dispersal, otherwise the 'rise' in the 1941 field would have been much greater.

It has already been noted that the flies show a marked reaction to wind changes, and additional evidence against the view that definite migration takes place is furnished by the following observations:

Mature females were recorded on the traps in reasonable abundance throughout the period in the bare part of the 1940 field.

Oviposition was observed in soil near the base of plants other than carrot, e.g. potato and *Atriplex* sp.

Uncompleted experiments to determine chemotropic reactions of the fly indicated that there does not appear to be any strong attraction displayed. Traps containing aromatic chemicals chosen as attractive to flies were placed in the hedge DE, along with some containing known repellents and controls. All failed to give any indication of attraction. The traps containing amyl acetate, benz-

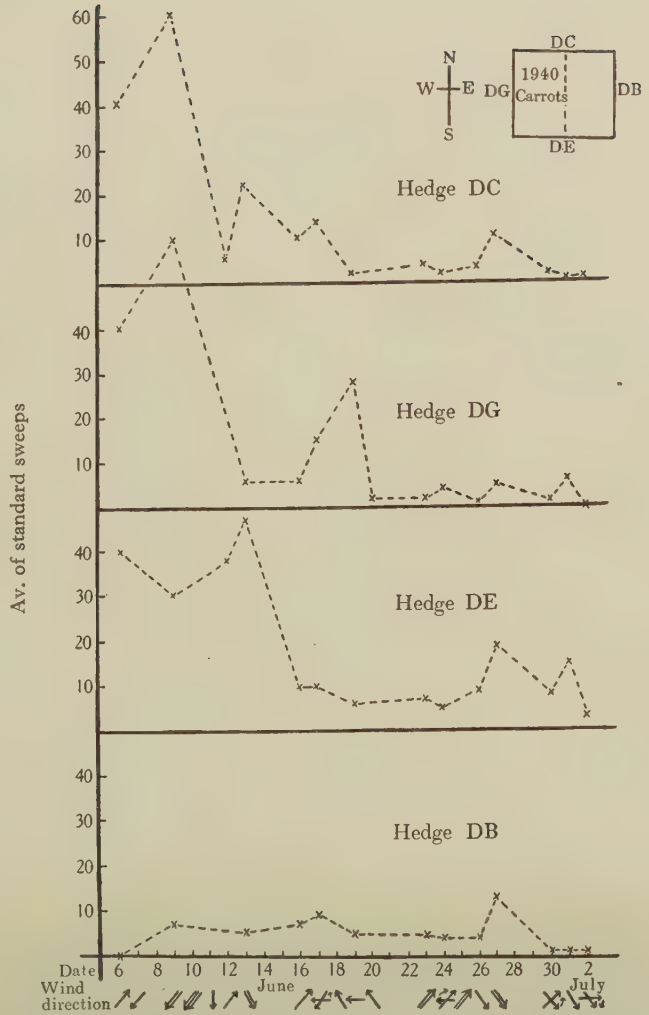

Fig. 5. Fluctuation in numbers of flies in hedges of 1940 carrot flies.

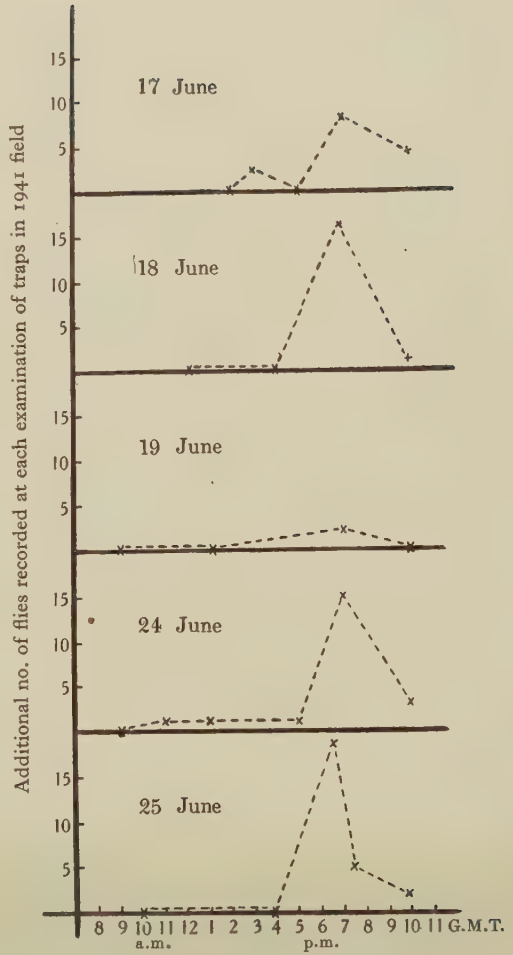

Fig. 6. Time of oviposition.

aldehyde and nitrobenzene did not catch appreciably more flies than the controls or even traps with naphthalene chosen as a repellent. The following figures were obtained:

	Nitro-benzene		Benz-aldehyde		Amyl acetate		Controls			Naph-thalene	
	A	B	A	B	A	B	A	B	C	A	B
No. of carrot fly caught on traps	6	4	7	6	5	12	4	17	7	7	—
Average	5·0		6·5		8·5		9·3			7·0	

Oviposition

From the absence of males and the high proportion of females with mature eggs (81 %) caught on the traps in the field, it was concluded that these figures gave some indication of the extent, time and duration of oviposition.

Method and place of oviposition. In general the observations confirmed those of K. M. Smith (1922). Females were observed ovipositing in the 1941 crop; typical observations are quoted below:

(i) 17 June, 2.55–3.05 p.m. (G.M.T.). Female carrot fly alighted on cotyledon leaf of carrot and remained at rest for a few seconds. It then explored the leaves of adjoining seedlings, finally resting head downwards on the original cotyledon leaf. The abdomen was jerked about and the ovipositor thrust out to its full extent five times. Then the fly moved and descended the stem but reappeared quickly. Short flights along 6 in. of the drill-row, followed by one short excursion away from the drill-row, were succeeded by the fly descending the stem of the same carrot seedling and disappearing out of sight down a crevice in the soil about ½ in. from the base of the stem. In this position it could not be observed; after some minutes it reappeared and flew away. Eggs were later found in the loose soil at the bottom of the crevice.

(ii) 17 June, 4.10–4.18 p.m. (G.M.T.). Female carrot fly settled on the leaf of a potato growing in the carrot drill-row and rested on the underside. It went down to a lower leaf where it rubbed the abdomen with the legs and extended the ovipositor several times. It then dropped on to the soil and walked to the main stem of the potato. Small carrots and some weeds were growing under the potato. It walked round the stem of an *Atriplex* sp. several times, halting after the third time. After a fourth round it immediately descended a crevice near the main stem of the potato. It was difficult to observe but became less agitated and was seen to clasp the stem with the front pair of legs and force the ovipositor into the loose, moist soil. It remained in this crevice for 5 min., moving round several times. On emerging it 'cleaned up' and flew away alighting on nearby carrot plants. The crevice was carefully examined and eggs noted in position. They were laid singly with the exception of two pairs—eleven ova were found. The whole process of oviposition occupied 8 min. In all cases observed the eggs were deposited down crevices in close proximity to seedling plants of various species. After the period of maximum oviposition, eggs could always be found in similar positions near the drill-row; interrow cultivation had disturbed the soil, removed weeds and resulted in conditions unsuitable for egg-laying between the rows.

Area of oviposition. The greater number of females caught on traps in close proximity to hedges with high populations indicated that egg-laying was most concentrated in these areas: 77·2 % were taken on traps A, A_1, B, B_1, C, C_1 near hedges with considerable populations of the fly (see Table 3). Mr Fisk stated that in previous years he had noticed that damage was most severe around the hedges, particularly where these were high and thick or where trees occurred, and in sheltered positions. This was further corroborated by observations that flies mainly undertook short trips from the hedge for oviposition. Subsequent search revealed the greatest number of eggs near the hedges. On 25 July it was noted that the only area of the field with numerous damaged plants was in the south-west corner, i.e. the area covered by the traps A, A_1, B, B_1, C, C_1 (see Fig. 2).

TABLE 3. *Number of flies on traps*

	1941 carrot field																						1940 field	
Date	A	A_1	B	B_1	C	C_1	D	D_1	E	E_1	F	F_1	G	G_1	H	H_1	I	I_1	J	J_1	K	K_1	Total	No. of traps	Total	No. of traps
13 June	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8	8	8	7
16 ,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	16	15	7	10
17 ,,	1	2	2	2	1	0	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	12	22	—	—
18 ,,	1	5	0	4	0	1	0	0	0	0	1	0	1	1	0	1	0	0	0	0	0	0	15	22	15	12
19 ,,	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	2	22	3	12
20 ,,	3	3	1	3	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	13	22	12	12
24 ,,	3	3	3	2	1	3	0	0	1	0	1	0	1	1	0	1	0	1	0	0	0	0	21	22	—	—
25 ,,	4	6	4	4	2	3	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0	26	22	—	—
26 ,,	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	22	—	—
30 ,,	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	22	3	22
1 July	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	3	22	4	22
2 ,,	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	22	1	22
Total	12	20	13	18	4	10	1	1	2	2	3	1	2	4	0	2	1	3	0	0	0	0	—	—	—	—

Note. 13 and 16 June: traps in 1941 field in experimental positions before final placing.
1940 field: traps varied in number and distribution and a continuous record was impossible owing to the cultivation of the field.

Period of oviposition. Unfortunately, it was not possible to fix the commencement of oviposition. On 13 June egg-laying was in progress on a considerable scale and continued with variations due to climatic factors until 25 June when a sudden decline occurred. From this date only a small proportion of the flies continued ovipositing. The results are summarized on Fig. 4.

Time of day for oviposition. Examination of the traps at frequent intervals fixed the variation in the number of flies out in the field during the day. These figures are shown on Fig. 6. Thus, the period of maximum oviposition may be fixed between 4 and 8 p.m. (G.M.T.).

Climatic factors and oviposition. Wind. The marked drop in the number of flies caught on 19 June (see Fig. 4) was due to a strong wind prevailing at the time of maximum oviposition and illustrated the influence of this factor (see also 'Effect of gale on local movement').

Temperature and light winds. High temperatures with little wind over the period 20–26 June were favourable for egg-laying.

Humidity. Oviposition did not appear to depend on high humidity. The period 20–26 June was definitely a time of low humidity but it was also a period of maximum oviposition.

Possibility of oviposition in bare ground. Traps set in the 1940 carrot field, which had been dragged and harrowed following the lifting of a section of the 1940 crop, showed that numbers of females were about in the fields for the whole of the period of oviposition indicated by the 1941 field (see Fig. 4): 81 % of these females contained mature eggs. No females were observed to lay eggs, and search for any possible eggs was greatly hampered by frequent cultivation of the field. The possibility of oviposition in bare ground was indicated but no evidence can be quoted to support this assumption.

SUGGESTIONS FOR CONTROL

It is vitally important that the first brood be controlled at its source. With the first brood on a site some distance from the current year's crop, the percentage of flies reaching this crop by wind dispersal may be small, but the success of this brood, however small, establishes the second brood in situ on the current year's crop. Therefore, by reducing the numbers at the source by control measures, the incidence of the first brood ovipositing

on the current year's crop will be reduced correspondingly. This will secure a minimum of damage by the second brood. It is, therefore, suggested that where hedges occur round the previous year's field some method of spraying could usefully be employed at about the peak period of assembly before general dispersal seriously affects large concentrations. Traps in the field indicated that the majority of flies return to the hedges at nightfall—as very few were caught before mid-afternoon. Therefore, spraying the hedges in the morning or late evening should give the greatest chance of success. The complete success of the method would depend on accurate information regarding the date of emergence.

Summary

Observations on the movement and oviposition of carrot flies in and near fields of carrots in north Lincolnshire lead to the following conclusions:

There is no evidence to indicate migration. Dispersal is general and is influenced by the wind. When the wind is *not too strong* some flies leave the hedges and when again seeking shelter tend to fly down wind. This results in a temporary spread in the direction in which the wind is blowing. The flies remain assembled in large numbers in the hedges surrounding the previous year's crop for a considerable period of time. Eggs are laid in $\frac{1}{2}$–1 in. crevices in proximity to the carrot drill-row. Greatest concentration of eggs is in close proximity to hedges holding large populations of the fly. The peak period of egg-laying was between 4 and 8 p.m. (G.M.T.).

REFERENCE

Smith, K. M. (1922). *Insect Pests of the Horticulturalist: their Nature and Control.* Vol. 1. *Onion, Carrot and Celery Flies.* London: Benn Bros.

(*Received* 16 *October* 1941)

STUDIES ON THE MECHANISM OF FUNGICIDAL ACTION

II. ELEMENTS OF THE THEORY OF VARIABILITY

By A. F. PARKER-RHODES, B.A.

University of Bristol Agricultural and Horticultural Research Station, Long Ashton, Bristol

The properties of the parameter called *variability*, having reference to a population of fungal spores differing in their tolerance to the toxic action of any compounds of a given element or radicle, are such as to render estimates of it of great use in the investigation of the mechanism of fungicidal action; certain of these properties will be examined in detail in this paper. In order that the theory shall be applicable to a given case, it is necessary to be satisfied that the toxic properties of the compounds investigated are, in fact, due to their containing the element which is common to them. It is thus most widely useful in investigating the toxicity of heavy metals and of such non-metals as sulphur, selenium, arsenic, etc. It is, however, equally possible to adapt the theory (though not necessarily the experimental procedure) to deal with beneficial effects, such as the absorption of rare elements essential to normal growth, or even to the operation of specific drugs.

Variability (using the term in its narrow sense as here defined) has not been used, as far as the writer is aware, by any investigator of fungicidal action, save in the first paper of this series (Parker-Rhodes, 1941), where restricted applications of the theory were made to certain compounds of certain metals.

Definition and properties of variability

Variability is defined as a parameter equal to the variance of the αth power of the variate, divided by the square of α and of the mean of the αth power of the variate, it being understood that the αth power of the variate is normally distributed. The *index of variation* is defined as the quantity α in the above definition; it is assumed that there exists a value of α such that the αth power of the variate is normally distributed.

The variability of x with index α is denoted by $W_\alpha(x)$, and the statistic thereof by $U_\alpha(x)$. The definition of variability may be expressed mathematically as follows:

$$W_\alpha(x) = V(x^\alpha)/\alpha^2\overline{x^\alpha}^2. \qquad (1)$$

If the variability of a variate xy be considered, where x^α and y^α are normally distributed and uncorrelated, the definition becomes

$$W_\alpha(xy) = V(x^\alpha y^\alpha)/\alpha^2\overline{x^\alpha y^\alpha}^2,$$

but
$$V(x^\alpha y^\alpha) = \overline{y^\alpha}^2 V(x^\alpha) + \overline{x^\alpha}^2 V(y^\alpha) + V(x^\alpha)\,V(y^\alpha),$$

therefore
$$W_\alpha(xy) = V(x^\alpha)/\alpha^2\overline{x^\alpha}^2 + V(y^\alpha)/\alpha^2\overline{y^\alpha}^2 + V(x^\alpha)\,V(y^\alpha)/\alpha^2\overline{x^\alpha}^2\overline{y^\alpha}^2$$
$$= W_\alpha(x) + W_\alpha(y) + \alpha^2 W_\alpha(x)\,W_\alpha(y). \qquad (2)$$

If we suppose in the above work that one of the quantities x, y is an invariant a, then $W_\alpha(a) = 0$, and hence

$$W_\alpha(ax) = W_\alpha(x). \qquad (3)$$

The above definition can only be applied for calculation of the variability from the variance if $\alpha \neq 0$; the limit of the variability as the index of variation tends to zero can be evaluated thus: expand the definition of variability as follows:

$$W_\alpha(x) = \frac{1}{n} \Sigma \left(\frac{x^\alpha - \overline{x^\alpha}}{\alpha \overline{x^\alpha}} \right)^2,$$

one term of which is the square of

$$\frac{x_s{}^\alpha - \overline{x^\alpha}}{\alpha \overline{x^\alpha}} = \frac{n x_s{}^\alpha - \Sigma x^\alpha}{\alpha \Sigma x^\alpha}$$

which is equal to

$$\frac{n}{\Sigma x^\alpha} \left\{ \left(\frac{x_s{}^\alpha - 1}{\alpha} \right) - \frac{1}{n} \left(\frac{x_1{}^\alpha - 1}{\alpha} + \frac{x_2{}^\alpha - 1}{\alpha} + \ldots + \frac{x_n{}^\alpha - 1}{\alpha} \right) \right\}.$$

The limit of the first term of this expression is $\log_e x_s$, and of the remainder

$$\frac{1}{n} \Sigma \log_e x = \overline{\log_e x};$$

returning to our original expansion, it follows that

$$\operatorname*{Lt}_{\alpha \to 0} W_\alpha(x) = \frac{1}{n} \Sigma (\log_e x - \overline{\log_e x})^2,$$

therefore

$$W_0(x) = V(\log_e x). \tag{4}$$

Estimation of the index of variation

Before proceeding to estimate the variability of a variate from a given set of figures, it is necessary to fix upon the index of variation. In the application of the theory to the study of fungicidal action, x represents the concentration of fungicide applied in solution, and we denote by q the proportional mortality, equal to $1 - p$, where p is the ratio of the percentage germination of the test spores to that found in controls germinating in distilled water. It follows that, if x^α is normally distributed (as is assumed in the preceding treatment), $y = b(x^\alpha - \overline{x^\alpha}) + \bar{y}$, where y is defined by

$$q = \int_{-\infty}^{y} \frac{e^{-\eta^2/2}}{\sqrt{(2\pi)}} \, d\eta;$$

y is called the 'probit mortality' (see Bliss, 1935), and its value can be determined from tables as given by Bliss, entering $100q$. α can therefore be estimated by plotting x^α against y for various values of α, and choosing that value giving the best straight line. As will appear later, however, we may often require to know whether two α-values determined for different toxic compounds are significantly different; to provide an approximate method of doing this, recourse is had to the method of maximum likelihood.

The likelihood for a given point defined by our observations (x, q) is equal to

$$q \log q' + p \log p',$$

where $p' = 1 - q'$ and p', q' are the values expected, assuming a given value of α. The total likelihood will be the weighted sum of these quantities for all the observed points (x, q). The condition that this sum should be a maximum is given by

$$\frac{dL}{d\alpha} = \frac{d}{d\alpha} \Sigma w (q \log q' + p \log p') = 0, \tag{5}$$

where the weighting coefficient for each point is $w = z^2/pq$, z being the ordinate of the normal curve.

But
$$q' = \int_{-\infty}^{y'} z'dy',$$

therefore
$$\frac{dL}{d\alpha} = \Sigma wz' \frac{dy'}{d\alpha}\left(\frac{q}{q'} - \frac{p}{p'}\right).$$

From the relation assumed between y', x, and α, since b must vary with α, we have

$$\frac{dy'}{d\alpha} = b\,(x^\alpha \log x - \overline{x^\alpha \log x}) + (x^\alpha - \overline{x^\alpha})\frac{db}{d\alpha};$$

but there is reason to believe that for values of α around the true one, $db/d\alpha$ is relatively small, so that the second term of $dy'/d\alpha$ may perhaps be neglected; thus, putting $f = \frac{z'^3}{p'^2 q'^2}$ and $\lambda = x^\alpha \log x - \overline{x^\alpha \log x}$ equation (5) becomes, approximately,

$$\frac{dL}{d\alpha} = \Sigma wz' b\lambda\left(\frac{q}{q'} - \frac{p}{p'}\right) = \Sigma fb\lambda\,(q - q') = 0$$

(for the sake of reducing the labour of computation we take f, for which a table can easily be compiled, instead of $(z^2z')/(pqp'q')$). The equation is then solved graphically, estimating the regression coefficient b by the ordinary methods; in this part of the work, however, it saves a great deal of computation if we take the weighting coefficients of the several points on the curve to be determined by their *observed* q's (which are independent of α), rather than by the values expected; this involves an error which makes the second differential of L *larger* than it otherwise would be.

Having estimated α thus, its standard deviation is equal to the reciprocal square root of $d^2L/d\alpha^2$, when observed quantities have been substituted for those expected in the expansion of this quantity. Now

$$\frac{d^2L}{d\alpha^2} = \Sigma\left\{(q - q')\frac{d}{d\alpha} fb\lambda + fb\lambda \frac{d}{d\alpha}(q - q')\right\},$$

on putting $q' = q$, the first term vanishes, so that

$$\sigma_\alpha = -1/\Sigma b^2\lambda^2 w^2. \tag{6}$$

This procedure involves three errors: (*a*) $\frac{db}{d\alpha} \neq 0$; (*b*) $f \neq \frac{z^2z'}{pqp'q'}$; and (*c*) the error introduced in the estimation of b mentioned above.

It follows from these errors that the values of α and σ_α thus obtained must be interpreted with caution. In order to obtain an estimate of their reliability, a set of figures relating to the toxicity of sodium dithionite to the spores of *Macrosporium sarcinaeforme* were taken and used to estimate α and σ_α by these methods; the distribution of x^α and of $x^{\alpha+2\sigma_\alpha}$ (where, according to the estimation, $\alpha = 0{\cdot}51$ and $\sigma_\alpha = 0{\cdot}13$) were tested for departure from normality by the use of the statistics $g_1\,(= m_3/m_2^{\frac{3}{2}})$ and $g_2\,(= (m_4 - 3m_2^2)/m_2^2)$, taking their standard deviations to be $\sqrt{(6/N)}$ and $\sqrt{(24/N)}$ respectively, where N was 250 (the number of spores counted for each value of x). It was found that x^α did not depart from normality, whereas $x^{\alpha+2\sigma_\alpha}$ did so by more than twice the standard deviation of the g's.

Thus, despite the errors involved, the method described may be regarded as justifiable as an *ad hoc* calculation, where different α-values have to be compared. (The figures used will be published in the next paper of the series.)

Estimation of variability

The definition of probit mortality y given in the preceding section was

$$q = \int_{-\infty}^{y} \frac{e^{-\eta^2/2}}{\sqrt{(2\pi)}}\, d\eta;$$

to make this tally numerically* with the probits as given in Bliss's Table I (1935), we must suppose that $\eta = x/\sigma$, where x is the normal deviate, and σ is its standard deviation. It follows then that, if x^α is normally distributed, $dy/dx^\alpha = 1/\sigma$; but $dy/dx^\alpha = b$. Therefore, according to equation (1), the variability is estimated by the statistic

$$U_\alpha(x) = 1/b^2\overline{x^\alpha}^2\alpha^2. \qquad (7)$$

In using this equation, we must bear in mind, first, that the regression coefficient b should be estimated using the *best* value of α: either that obtained by the method outlined above, or, in default of that, by inspection; and secondly, that $\overline{x^\alpha}$ here refers, as before, to the mean tolerance of the spores. It is *not* the same as the quantity denoted by this symbol in Bliss's paper. It is estimated by the statistic

$$m = \frac{\Sigma w x^\alpha}{\Sigma w} + \left(5 - \frac{\Sigma w y}{\Sigma w}\right)\Big/ b,$$

so that, using this formula, we may write (7) as $U_\alpha(x) = 1/b^2m^2\alpha^2$. This equation cannot be used if $\alpha = 0$; in this case (4) gives

$$U_0(x) = \left(\frac{d \log x}{dy}\right)^2 = \frac{1}{b^2}.$$

These statistics all refer to the *tolerance* of a given spore, defined as that concentration which just suffices to prevent that spore from germinating. It is not possible to measure the tolerance of a given spore, but this does not preclude the possibility of estimating parameters of its distribution. Whenever the variability of a quantity representing concentration is mentioned in the following work, it is understood to apply only to the tolerance of a spore.

Factors influencing variability

The influence of physical and chemical factors

Consider a solution of a single compound in concentration x_0, in which is immersed a sample of the given spore population. Let us suppose that the spore wall is an asymmetrically permeable membrane; this has not been demonstrated for any fungal spore, but that it is the case is almost certain from the structure of the spore wall, when this is compared with similar structures in other organisms, which have been shown to be asymmetrical (see Hurst (1941) for a discussion of this topic). Then, if the intropermeativity

* Bliss's probits have 5 added throughout.

of the given compound is β and the extropermeativity is β', the concentration x attained inside the spore after a lapse of time t will be given by the differential equation

$$\frac{dx}{dt}=\beta x_0-\beta' x, \tag{8}$$

from which

$$t=\int\frac{dx}{\beta x_0-\beta' x}+C$$

$$=C-\frac{1}{\beta'}\log(\beta x_0-\beta' x),$$

but $x=0$ when $t=0$, hence

$$C=\frac{1}{\beta'}\log \beta x_0,$$

therefore

$$t=\frac{1}{\beta'}\log\frac{\beta x_0}{\beta x_0-\beta' x},$$

therefore

$$x_0=\frac{\beta'}{\beta}(1-e^{-\beta' t})\,x; \tag{9a}$$

if we now suppose the experiment prolonged sufficiently for permeative equilibrium to be attained, this approximates to

$$x_0=\frac{\beta'}{\beta}\,x, \tag{9b}$$

so that

$$W_\alpha(x_0)=W_\alpha\left(\frac{\beta'}{\beta}\,x\right),$$

thus, using the relation (2) we have the result

$$W_\alpha(x_0)=W_\alpha(x)+W_\alpha\left(\frac{\beta'}{\beta}\right)+a^2W_\alpha(x)\,W_\alpha\left(\frac{\beta'}{\beta}\right). \tag{10}$$

The above treatment assumes that the compound supplied is itself capable of penetrating into the spore. If this is not the case, but the compound undergoes a reaction at the surface of the spore with some constituent of the spore secretion to give a resultant capable of penetrating, we must consider the effects of the chemical equilibrium established between the various reactants, thus: $MA+E\rightleftharpoons MB$, where E represents the reactant supplied by the spore. Putting $[MA]=z_1$, $[E]=a_1$, and $[MB]=y_1$, the law of mass action gives us the equation

$$z_1a_1=Ky_1. \tag{11}$$

If, as before, we denote the total concentration of MA supplied by x_0, we have that $x_0=z_1+y_1$, and thus, from (11), $x_0=z_1\left(1+\frac{a_1}{K}\right)$. But, at permeative equilibrium, from (9), $z=\frac{\gamma}{\gamma'}z_1$; $y=\frac{\beta}{\beta'}y_1=\frac{\beta}{\beta'}\frac{a_1}{K}.z_1$, where γ, γ' are the permeativities of MA. Now, putting x (total concentration inside the spore) $=y+z$, we get

$$x=z_1\left(\frac{\gamma}{\gamma'}+\frac{\beta a_1}{\beta' K}\right)=x_0\frac{K}{K+a_1}\left(\frac{\gamma}{\gamma'}+\frac{\beta a_1}{\beta' K}\right),$$

therefore

$$W_\alpha(x_0)=W_\alpha\left(x\,\frac{K+a_1}{K\left(\frac{\gamma}{\gamma'}+\frac{\beta a_1}{\beta' K}\right)}\right). \tag{12}$$

This expression requires further simplification before much use can be made of it; in particular, applying our supposition that the compound MA is non-permeative (or that its permeativity is negligible in comparison with that of MB) we can put $\gamma=0$, whereupon (12) becomes

$$W_\alpha(x_0)=W_\alpha\left(\frac{\beta'}{\beta}x.\frac{K+a_1}{a_1}\right)$$

$$=W_\alpha\left(\frac{\beta'}{\beta}x\right)+W_\alpha\left(\frac{K+a_1}{a_1}\right)+\alpha^2W_\alpha\left(\frac{\beta'}{\beta}x\right)W_\alpha\left(\frac{K+a_1}{a_1}\right). \tag{13}$$

Thus, provided the equilibrium does not lie very far on the side of the permeative compound (when $K\doteq 0$), the variability of the tolerance of a given population of spores will be greater to any non-permeative compound which can be converted into a permeative form than to any permeative one. If it cannot be converted into a permeative form under the conditions of the experiment, it will clearly be non-toxic.

By the same argument it can be shown that the introduction of each additional step required to bring the compound provided into a permeative form, in which any substance, the concentration of which in the zone of reaction is a variate of the spore population, takes part, will produce a further increase in the variability of the tolerance. Thus, suppose that a given metal can only be absorbed in the form of a co-ordination complex with some organic molecule present in the spore secretion; then the variability to the simple ion will be greater than that to the complex in question supplied ready-made, but the variability to a different complex may well be greater still, owing to the necessity of first breaking down this complex before building up the permeative one, supposing that the breakdown is facilitated by the spore secretion.

The influence of instability of the compound supplied

We shall assume for simplification that the compound decays according to a unimolecular law, thus: $x_0=X_0e^{-kt}$, where X_0 is the originally supplied concentration of the compound, and x_0 is the concentration left after time t. Thus equation (7) must be replaced by

$$\frac{dx}{dt}=\beta X_0e^{-kt}-\beta'x, \tag{14}$$

which can be solved by the use of the integrating factor $e^{\beta't}$, giving

$$d(e^{\beta't}x)=\beta X_0e^{(\beta'-k)t}\,dt,$$

therefore

$$e^{\beta't}x=\beta X_0\int e^{(\beta'-k)t}\,dt+C,$$

but when $t=0$, $x=0$, so that $C=\beta X_0/(k-\beta')$ and thus

$$x=\frac{\beta X_0}{k-\beta'}(e^{(\beta'-k)t}-1)/e^{\beta't}, \tag{15}$$

which approximates as time progresses to the form

$$X_0=\frac{\beta'-k}{\beta}e^{kt}\,x,$$

so that, for a given value of t (as must obtain in an actual experiment),

$$W_\alpha(X_0)=W_\alpha\left(\frac{\beta'-k}{\beta}x\right)>W_\alpha\left(\frac{\beta'}{\beta}x\right). \tag{16}$$

Thus, though the variability is increased if the velocity of decay is at all appreciable, we can be sure that if the apparent variability to a compound liable to this species of decay is *less* than that towards another stable one, the true variability will be yet more significantly less, but if it be the other way about, our conclusions are liable to error.

Influence of molecular constitution

Under this head will be considered the effect on variability of variations in the number of atoms of the effective element in the molecules of different compounds tested, of molecular dissociation and association, and so forth. Consider an experiment performed with a mixture of two compounds of a given element, neither of which is permeative to the spore, but which can combine together to give a new compound which is permeative (such a case, for example, is afforded by the sulphite ion, as will be shown in detail in a later contribution; this ion is not itself toxic, but two such, combining together to give an isomer of pyrosulphite, can in this form affect the spores). In the general case, we can represent the reaction thus: $AM+BM \rightleftharpoons CM_2$. If we denote the concentration of each of the three compounds present in equilibrium by y_1, z_1, and x_1, we have from the law of mass action, that $y_1 z_1 = Kx_1$; and the total concentration of M supplied, x_0, is equal to $y_1+z_1+2x_1$.

But in such an experiment, the mixture is diluted as a whole to different concentrations, so that the ratio $y_1 : z_1$ remains constant; we may therefore write $z_1 = ay_1$, and our equations can be reduced to

$$x_0 = (1+a)\, y_1 + 2x_1;\ a{y_1}^2 = Kx_1;$$

therefore

$$x_0 = (1+a)\sqrt{\left(\frac{Kx_1}{a}\right)} + 2x_1,$$

from which

$${x_0}^2 = (1+a)^2 \frac{Kx_1}{a} + 4\,(1+a)\, x_1 \sqrt{\left(\frac{Kx_1}{a}\right)} + 4{x_1}^2.$$

We have by definition that $W_\alpha(x^2) = V(x^{2\alpha})/d^2\overline{x^{2\alpha}}^2 = 4W_{2\alpha}(x)$, and therefore by an appropriate change in the index of variation we may put the result in the form

$$4W_{2\alpha}(x_0) = W_\alpha\left\{(1+a)^2 \frac{K}{a} x_1 + 4\,(1+a) x_1 \sqrt{\left(\frac{Kx_1}{a}\right)} + 4{x_1}^2\right\}. \tag{17}$$

Consider now the effect of different values of K. If the reactants are almost completely associated, the mixture is equivalent to the compound CM_2 itself, and as we should therefore expect, putting K very small gives

$$4W_{2\alpha}(x_0) = 4W_{2\alpha}(x_1), \tag{18}$$

whereas if K be very large (i.e. if the associated molecules are present only in a trace) the equation becomes

$$4W_{2\alpha}(x_0) = W_\alpha(x_1). \tag{19}$$

All these equations have meaning only if the reactants AM and BM are not permeative, whereas CM_2 is so. If K has a value intermediate between the very small and the very large, the variability will have an intermediate value also, though the precise form of the relationship is difficult to determine. Stating the result in words, we may say that a mixture of two compounds each containing a single atom of the effective element per molecule (or a single such compound, of which two molecules can react together) will give a variability one-quarter of that shown to any compound having two atoms to the molecule, and an

index of variation twice as great; or more generally, that the variability to any compound will be as the square of the number of molecules it contains, and the index of variation inversely as that number. The result holds equally for the dissociation of a non-permeative compound with two active atoms to the molecule into two parts or more, one of which (or both) is permeative, and for cases where the two active atoms are chemically different (e.g. two different metals).

However, in the case of a molecule dissociating into two or more parts, it is not necessary, for the above law to hold, that both parts should contain the active element, but only that one part of the molecule which does contain it should be permeative, but not the undissociated molecule.

The influence of synergism

The term *synergism* is used in pharmacology to designate the phenomenon of an increased toxicity of a mixture of poisons over the arithmetical sum of the toxicities of its components; the opposite effect being called *antagonism*.

Among the many causes of synergism, most of practical importance will have this property, that the magnitude of the synergic response will vary from one spore to another, and be, thus, a variate of the population. The occurrence of synergism of this kind will therefore inevitably increase the observed value of the variability, and it will be necessary in applying the theory of variability in practice to guard against this possibility.

The most likely case is that in which there is a synergic relation between some compound (perhaps itself non-permeative) and a product formed from it by the spore secretion. In view of the many unknown and perhaps unknowable factors in this situation, it is probable that we shall never be able entirely to exclude the possibility that our results may be distorted by this cause, but it should be remembered that most of the well-established cases of synergism are due to a sensitizing action of one poison upon an organism, rendering it more susceptible to attack by another. This situation is scarcely likely to arise as between closely related compounds of a single element, where, as we have every reason to believe, the toxic effect is due to some compound of the element formed only after its absorption by the spore, and of the same nature, in whatever form the element is actually absorbed.

Epistemological considerations

The meaning of equations involving variability

Consider such an equation as (13): this equation states that the variability, as measured from experimental observations, to a compound which cannot be absorbed by the spores directly is equal to the sum of three terms, the first of which is equal (according to (10)) to the variability to a compound which can be absorbed directly, the second to the variability of a certain characteristic of the spores, and the third to a function of the first two; if these three terms are all known, the equation tells us the value of the left-hand side. It is not an algebraic equation, for if we subtract, say, the third term from both sides, the result is meaningless. Instead of (13) we might equally have deduced from the algebraic equation preceding (12), putting $\gamma = 0$, the statistical equality

$$W_\alpha\left(\frac{\beta'}{\beta}x\right) = W_\alpha(x_0) + W_\alpha\left(\frac{a_1}{K+a_1}\right) + \alpha^2 W_\alpha(x_0)\, W_\alpha\left(\frac{a_1}{K+a_1}\right),$$

which indicates exactly the reverse of (13), namely, that the variability to the permeative compound is greater than to the non-permeative. The new form is incorrect, because when we use such an equation, we do so by comparing the *observed* value of the variability on the left-hand side with the value predicted by the equation; in order to make the prediction, we must be able to assign values to all the right-hand terms. In (13), we can evaluate the first term by using the hypothesis that some other compound which we have tested is the same as the product formed from the one now under test by the spores' secretion; the other two terms, though we cannot give them an exact value, must be positive; hence we predict that the variability of the compound under discussion will be greater than that other. If it is found otherwise, the hypothesis we made must have been false. But in the false version of (13) given above, we can only predict the value of $W_\alpha\left(\frac{\beta'}{\beta}x\right)$, which, *ex hypothesi*, we have already determined, from that of $W_\alpha(x_0)$, which has yet to be measured; furthermore, algebraic manipulation being inadmissible, this is the *only* 'use' to which such an equation can be put. The reader should satisfy himself that all the equations involving variability used above have, on the left-hand side, a quantity which we wish to predict in order to compare with observation, and on the right-hand side only quantities which, whether from previous observations or from some hypothesis which we wish to test, or both, can have values assigned to them.

Application of the theory to analysis of observations

It follows from the above that the logical form in which such an analysis can be made is that of the *reductio ad falsum*. A number of hypotheses are put forward concerning the chemical relations between the several compounds under comparison, from which, using the equations given by the theory, a series of relations can be predicted between the variabilities of the spores to each. Any such hypothesis can be held to be false if any one of the variabilities predicted from it differs significantly from that observed; and from the magnitude of the discrepancy the odds against the hypothesis can be computed. That member of a set of mutually exclusive hypotheses covering all the possibilities of the given case, which the experimenter deems tenable on other grounds, against which the odds are least may be named as the probably correct solution. If the odds against this are less than, e.g. 9 to 1, whereas the odds against its nearest rival are greater than, e.g. 19 to 1, it may with some confidence be asserted that this hypothesis represents the actual state of affairs.

It should be remembered, however, that the results presented above refer to parameters, W, whereas in practice one must needs deal with estimates of these, the statistics U. Thus, while (13) indeed indicates that the one variability will be greater than the other, it provides no guarantee that the difference will be significant when based on an actual set of data; if it is not so, it may well be that a more comprehensive experiment or a more careful technique would reveal a difference. This fact must be borne in mind in determining what tests of significance to apply to any given case.

Summary and conclusions

Variability is defined, and methods of estimation are outlined.

It is shown that the variability of a given population of spores to a compound which can penetrate the spore wall is less than to any other compound of the same element which

cannot do so unless it undergoes one or more reactions on the surface of the spore with substances secreted by it, and that the greater the number of such successive reactions that are required to bring it to a permeable form, the greater will the variability be.

It is shown that instability on the part of a given compound increases the apparent value of the variability of the spores to it.

It is shown that the variability of the spores to any compound is proportional to the square of the number of atoms of the effective element in a molecule of that compound, and that the index of variation is inversely proportional to that number, provided only one compound is permeative.

It is shown that if a molecule undergoes a very small dissociation into parts, some of which do not contain any of the active element, but of which some are permeative, whereas the undissociated molecule is not, then the variability to such a compound will be proportional to the square of the number of parts into which it dissociates, and the index of variation inversely proportional to that number.

It is shown that most kinds of synergism, occurring between constituents of a mixture used in an experiment, or between a compound under test and any product formed therefrom in the course of its toxic action, will interfere with the results by increasing the apparent value of the variability.

The method of applying these laws in the analysis of experimental results is discussed, and attention drawn to certain considerations with regard to the significance of mathematical expressions used in stating the results.

I have to thank Mr H. Todd, of the Mathematics Department, University of Bristol, for much valuable help in preparing this paper.

REFERENCES

Bliss, C. I. (1935). Calculation of the dosage-mortality curve. *Ann. appl. Biol.* **22**, 134.
Hurst, H. (1941). Insect cuticle as an asymmetrical membrane. *Nature, Lond.*, **147**, 388.
Parker-Rhodes, A. F. (1941). Studies on the mechanism of fungicidal action. I. Preliminary investigation of nickel, copper, zinc, silver, and mercury. *Ann. appl. Biol.* **28**, 389.

(*Received* 11 *November* 1941)

STUDIES ON THE MECHANISM OF FUNGICIDAL ACTION

III. SULPHUR

By A. F. PARKER-RHODES, B.A.

University of Bristol Agricultural and Horticultural Research Station, Long Ashton, Bristol

(With 2 Text-figures)

The controversy concerning the mechanism of the fungicidal action of sulphur and its compounds is still without any definite solution; the purpose of the experiments here reported was to attempt a solution as definite as possible in the case of *Macrosporium sarcinaeforme*, applying for this purpose the theory of variability expounded in the preceding paper of this series (Parker-Rhodes, 1942). Provided the chemical properties of the substances involved are such as to enable suitable experiments to be made, this theory allows of a definite answer being given to the following questions: (i) what compounds of sulphur can penetrate the spore wall? (ii) what other compounds can the spore secretion react with so as to produce permeative compounds? (iii) through what intermediate stages, if any, do such reactions proceed? The solution of the problem in the case of the spores of one species of fungus has no necessary relevance to any other species.

Certain Italian and French investigators, dealing with the use of sulphur against Erysiphaceae on vines, have concluded that elementary sulphur is the actual toxic agent (e.g. Sempio, 1932*a*, *b*). Among other reasons advanced for this view is that of the apparent 'action at a distance' exhibited by sulphur, it being supposed that the vapour of the element is effective. del Giudice (1931), however, working with the same species, regarded hydrogen sulphide as the active agent, produced by 'hydrogenation'; in this opinion he is supported by the work of McCallan & Wilcoxon (1931). From a comparison of the toxicities of various compounds the latter concluded that the necessary hydrogenation had to be accomplished either on the spore surface, or inside it, and they gave evidence that in the case of *Sclerotinia americana*, glutathione played an important role in the reactions. Young (1922) put forward the view that pentathionic acid was the toxic agent, but Wilcoxon & McCallan (1930) showed conclusively that the fungicidal characteristics of this compound were (with the fungi they studied) almost identical with those of sulphuric acid, the toxicity of both being due to $H^{\cdot}$-ions. Among others who have considered oxyacids responsible, Roach & Glynne (1928) gave evidence (based on a comparison of toxicities) that the effect of sulphur on *Synchytrium endobioticum* is exerted in the form of thiosulphuric acid, while Reckendorfer (1935), again with reference to the Erysiphaceae, concluded from chemical considerations that sulphur required oxidation before exhibiting its toxic action.

A number of investigators have stressed the importance of hydrolysis of sulphur as an essential stage in the toxication. Barker (1927) provided evidence that hydrogen sulphide formed in this way could act fungicidally, while Martin & Salmon (1932) found that not this compound, but polysulphides, were effective against Erysiphaceae. Martin (1930) pointed out that not only hydrogen sulphide, but also sulphoxylic acid, is produced in the

hydrolysis of sulphur, and may be fungicidal, a point difficult to prove experimentally, on account of the great instability of this compound.

Most of the above workers have studied the relative toxicities of various compounds. The toxicity of a given compound depends on its stability in the free state, its rate of reaction with the spore secretion, and possibly on its rate of penetration of the spore wall, as well as other factors. Thus, while it is fairly safe to assume that a non-toxic compound cannot be the agent of the effect, there is no reason why the compound actually responsible should be more or less toxic than any other. It is, indeed, well known to investigators of fungicides from the practical standpoint that the physical state of the preparation has a great influence on its toxicity. It is therefore unlikely that this method of approach can give a conclusive answer to the problem in question.

Experimental methods

Compounds investigated

The following compounds were studied for their effects on the germination of conidia of *Macrosporium sarcinaeforme*: (i) hydrogen sulphide, (ii) flowers of sulphur, (iii) sodium dithionite, (iv) sodium sulphite, (v) sodium dithionate, (vi) sodium tetrathionate, (vii) sodium thiosulphate, and (viii) sodium sulphate.

Methods of investigation

The compounds (iii)–(viii) above could be investigated in solution; two of these were tested at different *p*H values. Sodium sulphite solution was titrated with sulphurous acid (using B.D.H. Universal Indicator) so as to ascertain what proportion of the latter to add to obtain any desired *p*H; in this way solutions of sodium hydrogen sulphite were prepared having a *p*H of 5·6 and 8·3, these values being checked afterwards with the capillator. Sodium thiosulphate was tested in neutral solution and at *p*H 6·5; the latter just sufficed to bring about a temporary opalescence due to liberation of sulphur.

Sulphur was applied as a spray, suspended in water, using experimental methods already described for insoluble compounds (Parker-Rhodes, 1941). Hydrogen sulphide was applied to the spores in the form of a mixture with air, of known composition, prepared by means of a special apparatus; the spores were present in drops containing 0·04 ml. of aqueous suspension, five of which were carried on a slide (treated with cellulose nitrate) contained within a tube closed at each end by spring clips and rubber tubing. The apparatus is illustrated in Fig. 1. *A* and *B* are aspirators of about 1 and 4 l. capacity, *D* is a mercury manometer, *E* the tube containing the slide. 1 is a screw clip, 5 and 6 spring clips, 2, 3, 4, 7, 8, and 9 taps (3 and 7 being two-way) and 10 a water faucet connected to the mains. The water in *A*, and the mercury in the arm of *D* communicating with *A*, is covered with a layer of paraffin to prevent the gas dissolving in the water or contaminating the mercury.

The procedure in using the apparatus was as follows: (i) close 9; (ii) open 1 and 4; fill *A* with water through 10 and 3 till the paraffin reaches 4; (iii) close 4, and pass in a little more water till it reaches the level of 1, then close 10; (iv) open 2, and draw off a measured volume of water through 3 (the quantity being from 12·5 to 50 c.c. according to the concentration required), stopping the flow by closing 1, and only then close 3; (v) return pressure to atmospheric by cautiously opening 1, then close 1 and 2; (vi) open 4, draw off from 987·5 to 950 c.c. through 3, then close 4; (vii) attach *E* (with 5 and 6); (viii) open 6, 5, and 4, then cautiously opening 3 and 10 allow some 300 c.c. to enter *A*, thereby filling *E* with mixture by displacement, then close 10 and 3; (ix) close 6, 5, and 4, and detach *E*; (x) open 10 and 7 (*B* being empty), then open 8 cautiously and wait until pressure reaches a suitable value (commonly 36 cm. Hg above atmospheric), then close 10 and 8; (xi) open 3 expelling water from *A* till pressure reaches atmospheric, then close 3; (xii) repeat (vii)–(xi) as often as required. At end of work, (xiii) fill *A*, close 3, open 7, and when pressure in *B* no longer suffices to expel water, open 9.

By this means a geometric series of mixtures is obtained, the factor being $A/(36+A)$, where A is the atmospheric pressure in cm. Hg. The chief precaution required was to prevent the air in *B*

from becoming contaminated with hydrogen sulphide; to this end, care was taken never to open 8 unless the pressure in *B* was known to exceed that in *A*, and to open this tap slowly so as to avoid oscillations; and also it was advisable occasionally to flush *B* with water. Apart from this the main sources of error were (*a*) the impossibility of expelling gas from the whole of the effective volume of *A*, particularly the tube connecting *A* and *D*, and (*b*) a slight tendency for the gas to permeate the paraffin layer, and dissolve in the water. Together these two factors might cause an error of 2 % in the initial mixture.

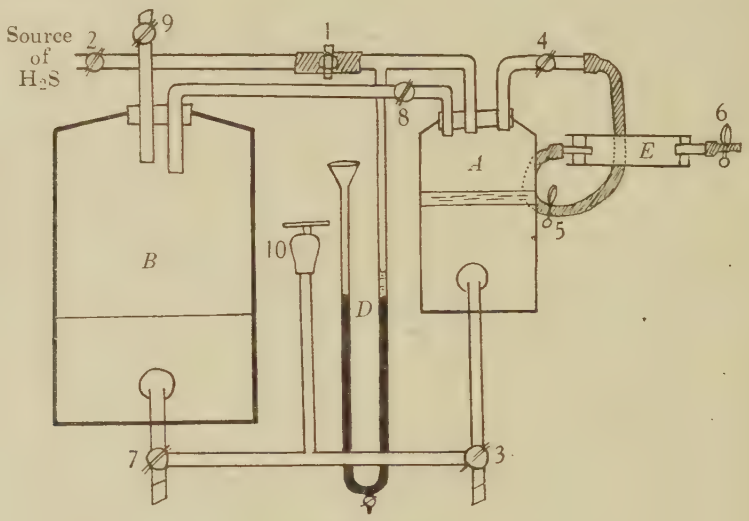

Fig. 1. For description see p. 137.

The rate of decomposition of hydrogen sulphide in the presence of water

The mixture when brought into contact with water tends to decompose by atmospheric oxidation, becoming yellowish, and ultimately depositing sulphur; to determine the rate of reaction, the following experiment was made. A saturated solution of hydrogen sulphide was prepared from the same kipp used as source in the apparatus described; 1 c.c. of this solution was withdrawn after measured intervals of time, diluted to 10 c.c., and titrated with *N*/100 potassium triiodide solution, using starch as an internal indicator. The following figures were obtained (Table 1):

TABLE 1

Hr. from start t	Normality x	$k=\frac{1}{t}\log\frac{x_0}{x_0-x}$
0·000	0·1731	—
0·617	0·1550	1·801
1·133	0·1463	1·483
1·967	0·1365	1·210
3·408	0·1097	1·341
5·633	0·0803	1·360
23·633	0·0096	1·221

Of the values of k the first is unduly large owing to loss by evaporation, and the last should be discounted because it includes the night period when the temperature dropped sharply; the mean of the remaining four figures (when the temperature remained close to 18° C.) is 1·348. It is evident that the reaction follows an essentially unimolecular law, under conditions which do not differ materially from those prevailing in a fungicidal test. The reaction between hydrogen sulphide and oxygen is usually given as $2H_2S+O_2\rightleftharpoons 2H_2O+S_2$, this being followed by:

$$H_2S+S_2\rightleftharpoons H_2S_3,\quad H_2S+H_2S_3\rightleftharpoons 2H_2S_2,\quad S_2+H_2S_3\rightleftharpoons H_2S_5$$

(see Bassett & Durrant, 1927). Since polysulphide ions react with iodine in the same way as hydrosulphide, it is not possible to ascertain from the above figures how much of the non-precipitated sulphur is present in each form, but there is undoubtedly an appreciable amount of polysulphide present.

Technique of germination counts

The method of preparing spore suspensions, and the details of counting germination, were in all respects the same as those described by Parker-Rhodes (1941). The cultures of *Macrosporium sarcinaeforme* used were direct descendants of those used in that work.

Experimental results

The results are summarized in Tables 2–7, where the value of x (concentration applied, in arbitrary units), q (proportional mortality), and n' (number of observations) are given; from these are calculated the quantities given in Table 8. Of these the first two columns give the values of the index of variation, as defined by Parker-Rhodes (1942) and estimated by the method therein described, and the standard deviation of this estimate; except in

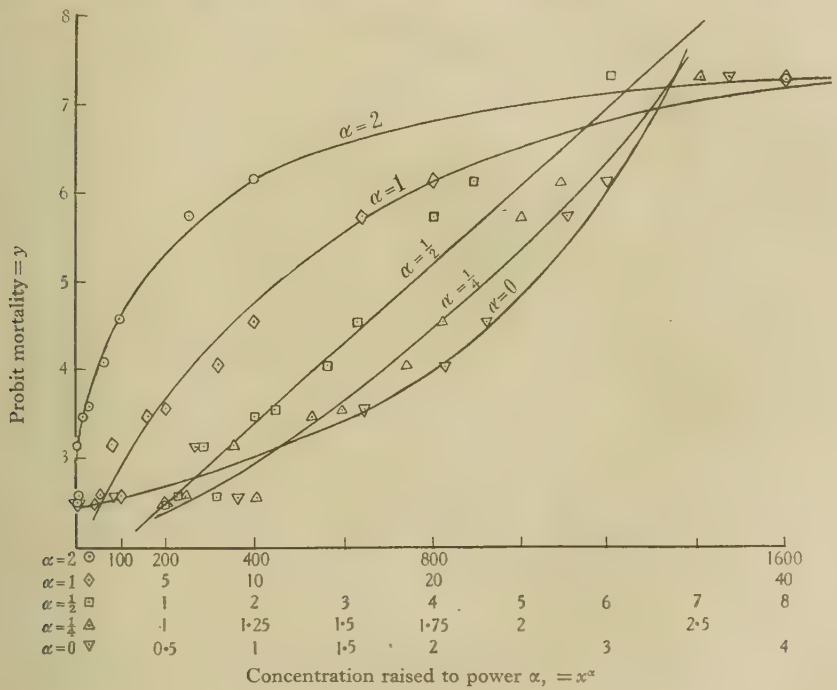

Fig. 2.

the case of alkaline sodium hydrogen sulphite solution, where the value had less theoretical importance, on account of which a graphical estimate was held to be sufficient. Such graphical estimates were required in all cases to determine the range of values on which to make the more exact estimate, the method of making which is illustrated in Fig. 2, showing the curves plotted from Table 5, from which it appears that $\alpha \simeq \frac{1}{2}$. The third column of Table 8 gives the values of α which present the closest approximation to the

estimated figures, given that their ratio shall be as 4 : 2 : 1, as expected on the basis of the theory of variability. The values for alkaline sodium hydrogen sulphite and for sulphur (the molecular composition of the hydrolysate being unknown) were omitted from the calculation. Column 4 contains the corrected values of the standard deviation. Column 5 gives the mean tolerance, calculated from the formula

$$m = \frac{\Sigma(wx^{\alpha})}{\Sigma(w)} + \frac{5 - \Sigma(wy)/\Sigma(w)}{b},$$

w being the weighting coefficient for each point, y the probit mortality determined from q (see Bliss, 1935), and b the regression coefficient of y against x^{α}. For this purpose the value of α given in column 3 was used. In the case of hydrogen sulphide, the instability of the

TABLE 2. *Hydrogen sulphide*

x	q	n'
1·00	0·000	8
2·49	0·030	10
3·92	0·068	10
6·18	0·237	10
9·75	0·826	10

Unit of x indeterminate

TABLE 3. *Sodium hydrogen sulphite (acid)*

x	q	n'
1·00	0·029	10
1·10	0·051	10
1·34	0·037	10
1·63	0·089	10
1·79	0·111	8
1·96	0·128	10
2·35	0·252	8
2·38	0·142	10
2·82	0·385	10
3·17	0·535	10
4·23	0·670	8
5·63	0·930	6

Unit of x: 0·00071 g.-atom S/l.

TABLE 4. *Sodium hydrogen sulphite (alkaline)*

x	q	n'
1·00	0·227	10
1·34	0·454	8
1·79	0·642	6
2·38	0·748	9
3·17	0·921	10
4·23	0·9959	10
5·63	1·0000	10

Unit of x: 0·00134 g.-atom S/l.

TABLE 5. *Sodium dithionite*

x	q	n'
1·00	0·006	8
1·25	0·008	10
2·00	0·032	10
2·50	0·008	10
4·00	0·064	10
5·00	0·075	10
8·00	0·178	10
10·00	0·323	10
16·00	0·773	10
20·00	0·876	10
40·00	0·9911	10
80·00	1·0000	10

Unit of x: 0·000782 g.-atom S/l.

TABLE 6. *Sodium tetrathionate*

x	q	n'
1	0·048	8
5	0·115	10
16	0·436	10
40	0·641	10
80	0·804	10
150	0·915	10

Unit of x: 0·000281 g.-atom S/l.

TABLE 7. *Sulphur*

x	q	n'
1·0	0·051	12
1·4	0·168	12
2·0	0·089	6
2·8	0·386	12
4·0	0·398	12
5·6	0·518	12
11·3	0·818	12
16·0	0·979	12
22·7	0·972	12
45·3	0·9827	12

Unit of x indeterminate

TABLE 8. *Summary of results*

	Compound tested	Index of variation: Estimated α	Estimated σ_α	Adjusted α	Adjusted σ_α	m	M‡	Variability U
2	hydrogen sulphide	0·90	0·71	0·95	0·36	6·89	0·6596	0·104
3	sodium hydrogen sulphite (acid)	0·82	0·32	0·95	0·36	3·20	0·3143	0·154
4	sodium hydrogen sulphite (alkaline)	< 1	—	0·95*	—	3·44	0·7834	0·444
5	sodium dithionite	0·51	0·13	0·48	0·18	3·32	0·4585	0·272
6	sodium tetrathionate	0·28	0·17	0·24	0·09	1·92	0·3217	1·92
7	sulphur	0·38	0·22	0·38†	—	1·85	0·2181	0·78

* This was the value assumed in calculating U, though it was not used in the adjustments applied to the α-values.

† The α-value for sulphur was not used in the adjustments, for reasons stated in the text above, but was used unadjusted in calculating U.

‡ From statistical considerations to be published shortly it is probable that the figures in this column ought to be somewhat lower than as here given.

compound prevents any meaning being attached to this statistic in terms of toxicity, as also with the insoluble elementary sulphur, but for the other compounds used the mean tolerance (= L.D. 50) can be calculated from the values given in each table for the arbitrary units used to measure x.

Table 8, column 6, gives the values of $M = m^2/(\Sigma\,(wx^{2\alpha}) - \bar{x}^{\alpha}\Sigma\,(wx^{\alpha}))$, which is a quantity used in calculating the significance of differences in the variability U given in the last column. $U = 1/\alpha^2 m^2 b^2$, and the criterion for significance at the 5 % level is that

$$\frac{U_1 + U_2 - 2\sqrt{(U_1 U_2)}}{U_1 U_2\,(\alpha_1{}^2 M_1 + \alpha_2{}^2 M_2)} \geqslant 3{\cdot}84.$$

Using this criterion, the following significant differences are found:

$H_2S < Na_2S_2O_4 < S < Na_2S_4O_6$; $H_2S \not< NaHSO_3$ (acid) $\not< Na_2S_2O_4$; acid $\not<$ alkaline $NaHSO_3$; there were no significant differences in the values of $\alpha^2 U$.

In addition to the compounds referred to in the tables, the following were tested and found to be without any toxic properties at 1/20 g.-atom S/l.: sodium sulphate, sodium thiosulphate (both in acid and neutral solutions), sodium dithionate.

Discussion

To avoid redundancy, a knowledge of the matter of the second paper of this series, on the *theory of variability*, will be assumed throughout this section (Parker-Rhodes, 1942).

It will be observed that there is no significant departure from the prediction of the theory, that the index of variation of a given population of spores to compounds of a given element will vary inversely as the number of atoms of the element present in the molecule, provided that permeative molecules have all the same number of atoms of the active element. The theory also predicts that, with the same proviso, the variabilities will vary as the square of the number of atoms. That there are significant differences between the values of U in the different categories, whereas no such differences exist as between the values of $\alpha^2 U$, provides more positive evidence in support of the theory, and at the same time indicates that all permeative compounds have, in fact, the same number of sulphur atoms in the molecule.

In determining this number we may first eliminate a large number of possible permeative compounds or ions owing to the fact that acid sodium thiosulphate solution is non-toxic. In this solution there exist, according to Bassett & Durrant (1927), besides the ions S_2O_3'', the following: HS', HSO_3', O_3SSSO_3'', $O_3SS_2SO_3''$, $O_3SS_3SO_3''$, pyrothiosulphate, and elementary sulphur. This virtually eliminates all ions with more than two sulphur atoms, and of those having only one atom leaves only HSO_2' and SO_4''; of these the latter is eliminated by the non-toxicity of sodium sulphate, while the former appears very improbable from the low value of α for sulphur suspension, in which HSO_2' is almost certainly present. It is probable, therefore, that any permeative ion will have two atoms of sulphur to the molecule. The only ion of this type likely to be present appreciably in 'hydrogen sulphide' solution is HS_2'. Sodium dithionite consists almost entirely of such anions with Na^+, and sodium sulphite contains in equilibrium small numbers of pyrosulphite ions.

Bassett & Durrant (1927) give evidence that pyrosulphite can readily be transformed by autoreduction and auto-oxidation to sulphate and dithionite, the action being reversible; it is therefore not unlikely that there should be no appreciable difference in the variability

of the tolerance of the spores to these two ions, as is indicated by the similarity of the values of $\alpha^2 U$. Actually, however, the value for sodium hydrogen sulphite is more than twice that for sodium dithionite, so that, if there is a difference, it is likely to be that the latter is permeative, whereas the former is not. It is more surprising that there should be no difference between these ions and the hydrogen sulphide solution, since the ion HS_2' is both chemically and physically quite different from HO_2SSO_2' (dithionite). Three explanations suggest themselves: (*a*) that the two ions are easily interconvertible, (*b*) that they are both permeative, or (*c*) that the only permeative ion is an isomer of S_2O_2 (thiosulphite), into which HS_2' may be transformed by oxidation under the influence of the spore secretion, and HO_2SSO_2' by reduction. Hypothesis (*a*) is clearly very unlikely. There is at present no means of distinguishing between (*b*) and (*c*), and in view of the great instability of the thiosulphite ion, it does not seem possible to devise an experiment which would make the distinction.

We may therefore conclude that only certain sulphur ions, containing two sulphur atoms to the ion, can penetrate the spore wall of *Macrosporium sarcinaeforme*, hydrodisulphide, dithionite, and perhaps pyrosulphite, being among their number; or if only one such ion is permeative, it is thiosulphite. That any ion or compound capable of being transformed by association, decomposition, oxidation, or reduction, or any combination of such reactions, into one of the above will have toxic properties for the spores, but that any ion or compound not so capable will be non-toxic.

Concerning the action of elementary sulphur, we may assume that some ion having two sulphur atoms is the active agent; putting $\alpha = 0{\cdot}48$, we find that the variability to sulphur is greater than that to sodium dithionite by a significant amount; according to theory, this means that the active agent (or at least the principal active agent) requires to undergo some reaction influenced by the spore secretion in order to be converted into a permeative form. According to the scheme proposed by Bassett & Durrant (1927) pyrosulphoxylate is the first ion of the type required to be formed; this is an isomer of thiosulphate and so is probably present in acid thiosulphate solution. From the non-toxicity of this solution it can be inferred that this ion is not itself permeative, which agrees with our former conclusion and suggests that it requires either oxidation to dithionite or reduction to thiosulphite before being absorbed.

It is thought by some (see Bassett & Durrant, 1927) that there exist two isomeric forms of certain ions containing two sulphur atoms, a yellow form favoured by acid conditions and a colourless form present in alkaline solution. Since the theory requires that there should exist only a trace of associated molecules in solution, for the variability to have the expected value, the figures for acid and alkaline sulphite suggest that only the colourless form can be absorbed.

Additional support for the values of α estimated, as given in the Tables, accrues from the figures of McCallan & Wilcoxon (1931) for the action of hydrogen sulphide on *Macrosporium sarcinaeforme*, which indicate that the logarithmic law assumed by them is much less in accordance with the facts than a higher value of α. Their figures for the action of other compounds are too sketchy to allow of α being estimated, but their figures for the action of this same compound on *Sclerotinia americana* indicate an index of variation between $\frac{1}{2}$ and 1.

Summary

The reaction of spores of *Macrosporium sarcinaeforme* to a selection of sulphur compounds, including elementary sulphur, was studied. Details are given of the methods used with hydrogen sulphide, which involved examining the behaviour of the spores in a static atmosphere containing the gas.

Figures are given relative to the rate of decay of hydrogen sulphide in aqueous solution under the conditions of the experiment, due to atmospheric oxidation, which indicate that the reaction is effectively unimolecular.

From the results, it is inferred that sulphur can be absorbed by the spores only in the form of the ions hydrodisulphide, dithionite, or pyrosulphite, or else only as thiosulphite; of the yellow and white isomers of the ions, it is thought possible that only the latter can be absorbed. It is considered probable that in the given case sulphur exerts its effect indirectly through the pyrosulphoxylate ion present in the hydrolysate. Agreement of the results with the theory of variability is noted.

My thanks are due to Dr H. Martin for help in preparing the paper, and to Prof. B. T. P. Barker for permission to work in his laboratory.

REFERENCES

Barker, B. T. P. (1927). Investigations on the fungicidal action of sulphur. *Ann. Rep. of the Agricultural and Horticultural Research Station, Long Ashton*, p. 72.

Bassett, H. & Durrant, R. G. (1927). The interrelationships of the sulphur acids. *J. Chem. Soc.* p. 1401.

Bliss, C. I. (1935). Calculation of the dosage-mortality curve. *Ann. appl. Biol.* **22**, 134.

del Giudice, E. (1931). Alcune esperienze sull' azione anticrittogamica dello zolfo. *Boll. Staz. Pat. veg. Roma* (N.S.), **11**, 128.

McCallan, S. E. A. & Wilcoxon, F. (1931). The fungicidal action of sulphur. II. The production of hydrogen sulphide by sulphured leaves and spores, and its toxicity to spores. *Contr. Boyce Thompson Inst.* **3**, 13.

Martin, H. (1930). The hydrolysis of sulphur in relation to its fungicidal activity. *J. agric. Sci.* **20**, 32.

Martin, H. & Salmon, S. (1932). Fungicidal properties of certain spray fluids. IX. Fungicidal properties of products of the hydrolysis of sulphur. *J. agric. Sci.* **22**, 595.

Parker-Rhodes, A. F. (1941). Studies on the mechanism of fungicidal action. I. Preliminary investigations on nickel, copper, zinc, silver and mercury. *Ann. appl. Biol.* **28**, 389.

—— (1942). Studies in the mechanism of fungicidal action. II. Elements of the theory of variability. *Ann. appl. Biol.* **29**, 126.

Reckendorfer, P. (1935). Die chemische grundlage der fungiziden wirkung des weinbergsschwefels. *Z. PflKrankh.* **45**, 537.

Roach, W. A. & Glynne, Mary D. (1928). The toxicity of sulphur compounds to *Synchytrium endobioticum*, the fungus causing wart disease of potatoes. *Ann. appl. Biol.* **15**, 168.

Sempio, C. (1932*a*). Il meccanismo di azione dello zolfo nella lotta contro le erisifacee. *Ann. Tec. agr. Roma*, **5**, 4.

—— (1932*b*). Sulla interpretazione dello meccanismo intimo di azione dello zolfo come anticrittogamica. *Mem. R. Accad. Ital.* 30 pp.

Wilcoxon, F. & McCallan, S. E. A. (1930). The fungicidal action of sulphur. I. The alleged role of pentathionic acid. *Phytopathology*, **20**, 391.

Young, H. C. (1922). The toxic property of sulphur. *Ann. Mo. bot. Gdn.* **9**, 403.

(*Received* 11 *November* 1941)

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

ORDINARY MEETING of the Association of Applied Biologists held in the London School of Hygiene and Tropical Medicine, on Friday, 10 October 1941, at 11 a.m., the Chair being occupied by the President, Dr Hubert Martin.

SYMPOSIUM ON WIREWORM INVESTIGATIONS

The following papers were read:

Investigations on wireworms: introductory. By J. C. F. FRYER, M.A. (Ministry of Agriculture and Fisheries).

Wireworms and crop production. By S. G. JARY, B.A. (South-Eastern Agricultural College, Wye).

The analysis of wireworm populations. By G. SALT, M.A., and F. S. J. HOLLICK, M.A. (University of Cambridge). *Paper to be published in a later number of the Annals of Applied Biology.*

Statistical problems in field sampling for wireworms. By F. YATES, Sc.D., and D. J. FINNEY, M.A. (Rothamsted Experimental Station).

Observations on some factors influencing growth in wireworms of the genus *Agriotes* Esch. By A. C. EVANS, Ph.D. and H. C. GOUGH, Ph.D. (Rothamsted Experimental Station).

Wireworms and agriculture, with special reference to *Agriotes obscurus* L. By HERBERT W. MILES, D.Sc. (University of Manchester).

Observations on the biology of *Agriotes obscurus* L. I. The adult insect. By MORRIS COHEN, Ph.D. (University of Manchester).

INVESTIGATIONS ON WIREWORMS: INTRODUCTORY

BY J. C. F. FRYER, M.A.

Ministry of Agriculture and Fisheries

FROM the agricultural aspect, the importance of wireworms arises from three well-known facts. First, wireworms, at all events those of the most abundant genus—*Agriotes*—are essentially inhabitants of grassland, or possibly it would be better to say of a mixed plant population in which members of the Gramineae predominate. It is true that wireworms can and do persist in land which is more or less permanently arable (possibly because corn crops provide conditions sufficiently resembling grassland to attract the beetles when egg laying), but this aspect of the problem is of less immediate concern. Secondly, Elaterid larvae in general, and wireworms in particular, spend a long time—something like 5 years—

in the larval stages. Thirdly, wireworms are able to feed on a wide range of important crops, notably cereal crops.

From this it follows that the wireworm problem is likely to be serious in any system of rotation in which grassland alternates with arable, assuming that the grass is down for long enough to become heavily infested, while it will be still more serious when there is any major change from grass farming to arable farming. The importance of wireworms as pests turns therefore on the trend of agricultural practice; when this is to lay down arable to grassland their importance diminishes, while it increases when the reverse takes place.

In time of war, the urgent need to secure the maximum production of food necessitates the greatest possible acreage of land in corn and other arable crops, and since the periods preceding both the present and the previous war were characterized by the laying down of arable to grass on a vast scale, the reconversion to arable has had to be on a scale no less vast. In such circumstances the wireworm problem, normally of an importance comparable with that of many other agricultural pests, suddenly becomes a major issue owing to the very large acreage potentially affected and the fact that crop losses can only be made up with difficulty, if at all, by imports from overseas. The present importance of the problem therefore needs no emphasis, and the next question concerns the measures that can be taken to meet it. To appreciate this aspect of the matter, it is necessary to refer to the experience of the last war.

Agricultural entomologists were then few in numbers and no concentration on the wireworm problem was possible. All that could be done was to observe the results being obtained in practice and to make any obvious deductions from them. This work could not be placed on any proper statistical basis and there is no full record of it available. Nevertheless, it provided a good deal of information, often in confirmation of the results of earlier workers, that has stood the country in good stead in the present crisis. For confirmation of this, readers should refer to Growmore Leaflet No. 11 on *Pests and the Breaking of Grassland*, written in September 1939, but one or two points arising from the experiences of the last war are worth emphasizing.

In the first place, the importance of cultivation and manuring in relation to wireworm attack, notably on cereal crops, stood out above everything else. Although there were many instances in which wireworms were solely responsible for crop failures, nevertheless it was more often found that such failures were due to a combination of factors acting together, each of which alone might have caused crop depression but not complete losses on the scale experienced. Secondly, considerable additional experience was gained as to the crops which are partially resistant to wireworms and can therefore be grown in the face of infestations that would cause the complete destruction of cereal crops. The observation that pulse crops such as peas and beans, and flax—all important in war time—were resistant to attack was specially useful. Thirdly, it became evident that not all grass fields were affected by wireworms, and so far as could be judged by the damage to the ensuing crop, the degree of infestation varied within wide limits.

Unfortunately, at the time no co-ordinated attempt was made, or indeed could have been made, to define wireworm populations in actual numbers so as to correlate crop resistance and susceptibility with the different levels of population. It is to the credit of one of the Advisory Entomologists, Mr A. Roebuck, of the Midland Agricultural College, that even in the circumstances of the last war he realized the importance of enumerating wire-

worm populations, and on the basis of soil samples estimated the numbers of wireworms per acre occurring in some eight fields under observation.

The only other work on wireworms that arose directly out of the war of 1914–18 was that of Rymer Roberts, which was concerned with the biology and morphology of wireworms and is well known to members of the Association.

With the end of the last war, and after a brief spell of prosperity, the economic conditions of farming deteriorated rapidly and resulted in the conversion of arable land to grass on a scale never before experienced. The wireworm problem in agriculture, if not forgotten by the entomological world, was at least pushed into the background by the necessity of tackling problems concerning those crops that were profitable, mainly in the horticultural field. In intensive cultivation, however, which often makes use of the accumulated fertility in grassland or 'top spit', the wireworm problem still was of importance, and reference may be made to the method worked out by Petherbridge & Miles of using wheat as a bait in conjunction with calcium cyanide for killing wireworms under glass and also to the use of wheat by the former investigator as a trap crop for preventing wireworm attacking sugar beet, one of the few agricultural crops which survived in a condition of relative prosperity.

With the exception of these two investigations, which were of a special character, no general attack on the wireworm problem was made during the period after the last war until 1936, when the Cheshire and Lancashire farmers realized that they were suffering appreciable losses both to cereal and potato crops, and as a result asked the University of Manchester to intensify work on the problem. The loss on potato crops in 1936 in Cheshire was estimated at not less than £100,000, and losses of this character were sufficient to persuade the National Farmers' Union, the Potato Marketing Board, the County Councils and Agricultural Societies of Lancashire and Cheshire to join with the Ministry in financing an investigation dealing specially with the problem in the two counties concerned. A small field station at Warburton in Cheshire was acquired, and work under the direction of Dr H. W. Miles has proceeded since 1937. The investigations were directed mainly to the study of the biology and ecology of the pest under field conditions, and the basic question which it was desired to answer was why there should be such variation in the numbers of wireworms in fields which had all been farmed under the same general system, for it was felt that an answer to this might provide a solution to the wireworm problem.

One other investigation of a general character was the work at Rothamsted on soil insecticides, for although it was not concerned specifically with wireworms, nevertheless the fact that these pests were used as test subjects, and that their control was one of the chief objects of the search for better soil insecticides, implied that much general information regarding wireworms would also be obtained.

As a whole, however, while the work carried out in the interim between the two wars had led to an increased knowledge both of the biology and morphology of wireworms, it had not resulted in any new and easy method of dealing with the pests under agricultural conditions, and the same can be said of similar work carried out overseas. With the approach of the second war, therefore, it became necessary to consider what use could be made of existing knowledge, having regard to the prospect of another 'ploughing up' campaign. A conference of Advisory Entomologists and others specially interested in wireworm research was therefore held in December 1938, and it was agreed that by the use of measures already known, e.g. by the choice of suitable crops and methods of cultivation, the loss

from wireworms could be greatly reduced provided the measures were selected in relation to the dimensions of the wireworm populations present. It was considered that by soil sampling methods an estimate of the wireworm population in any field could be obtained, and that if a rough estimate were sufficient, as seemed probable, the sampling of fields on quite a big scale might be a practical proposition. It was also agreed that if accurate information as to the effects of known wireworm populations on different crops in different districts could be obtained, advice on the agricultural treatment to be given in specific cases could be placed on a firmer foundation than would otherwise be possible.

With the help of Dr Yates and Mr Finney of the Rothamsted Experimental Station on the statistical aspects of the problem, a co-operative scheme of work was drawn up to be carried out at as many of the Advisory Centres as possible. This scheme was defined as having three objects:

(1) To find a method of estimating the wireworm population of a grass field with sufficient accuracy and sufficient speed to be of service in advisory work.

(2) To obtain some guide as to the damage to be expected from wireworm populations of different sizes.

(3) To discover any obvious correlations between the size of the wireworm population in any field and other factors, such as soil type or cultural treatment.

Work was begun in the early months of 1939, and approximately 100 grass fields about to be broken were investigated. During this initial period, attention was chiefly given to technique in relation to the size and number of samples that would have to be taken. As a result, Dr Yates and Mr Finney obtained sufficient material to advise that with fields up to the size of about 20 acres the examination of twenty cores of soil of 4 in. diam., taken to a depth at which wireworms were to be found, would provide an estimate of the population that would probably be sufficient for advisory work. This finding was confirmed by further work carried out before the harvest of 1940, by which time an additional 473 fields had been surveyed. The statistical aspect of the work is dealt with elsewhere, and it is sufficient to say here that although the sampling error, regarded from the research point of view, was large, nevertheless the results from the advisory standpoint seemed reasonably adequate and enabled the wireworm population of a field to be placed in one of four categories—slightly infested, with a population not much exceeding 300,000 per acre; moderately infested, ranging from 301,000 to 600,000; highly infested, from 601,000 to 1,000,000; and very high populations of over a million.

As to the effects of wireworm populations on the crops sown after the grass had been ploughed, a programme involving plant counts and sampling for yield was also carried through, and some of the results are discussed in subsequent communications. Nearly half of the fields sampled when in grass were sown to oats, and when these crops were divided into 'successful crops' (two-thirds of the average yield or over), 'poor crops' (from two-thirds to one-third), and 'failures' (under one-third of an average crop), the distribution in relation to wireworm populations was as follows:

Wireworm population	Successful crops	Poor crops	Failures
Under 300,000	103	12	2
301,000–600,000	31	5	9
601,000–1,000,000	11	7	9
Over 1,000,000	4	6	11

Precisely similar trends were obtained with wheat and dredge corn, but barley suggested no obvious correlation. Other useful information was obtained, such as that good crops of peas and beans could be grown in face of wireworm populations exceeding 1 million/acre, and that flax, although highly resistant to attack, was not wholly immune.

The third object of the work was to discover any obvious correlations between the size of the wireworm population in any field and other factors, such as soil type and cultural treatment. The object here was to obtain some clue to the problem of why one field should be heavily infested and another in the neighbourhood, even perhaps an adjoining field, should contain a much smaller wireworm population. On this problem the co-operating workers recorded for each field a number of particulars, such as nature of soil, drainage, nature of grassland, whether hayed or grazed, and date of ploughing. The first point that emerged was that, regardless of soil type, the average size of the wireworm populations decreased from the south of the country to the north, and to some extent from east to west. This does not mean that from the agricultural point of view the wireworm problem is unimportant in the north-west, for instance, for in practically every county there are sufficient highly infested fields to lead to crop failures if incorrectly treated.

Next, as to the influence of soil type: although in most agricultural provinces it is possible to pick out areas in which high or low populations are specially frequent, and often those areas coincide approximately with certain soil formations, any attempt at generalization would be premature. From the practical point of view, however, the observations are valuable in showing the districts in which wireworm sampling from the advisory standpoint gives the greatest return.

With other factors, the results were even less conclusive than they were with soil characteristics. Age of grassland, provided it exceeded 2–3 years, appeared to have no bearing on infestation, nor did treatment in respect of whether it had been mown or grazed. One belief often held in the past, namely, that poor rough grass was more heavily infested than good pastures, was largely discredited.

In normal times it would clearly have been desirable that these exploratory phases of the work should have been continued for another year or two, and probably have been reinforced by definite experiment. In particular, it would have been desirable to concentrate on the effects of wireworm populations on different crops the second and third years after grass, because most of the information already gathered concerned first crops after grass. But enough information had accumulated to show that a knowledge of the wireworm population of grass fields was exceedingly valuable in planning their subsequent treatment and cropping. In the interests of food production, therefore, the research aspects of the scheme had to give way to the advisory, and the staffs of the different centres were expanded so as to enable a substantial acreage to be dealt with. The total staff employed is over seventy, and it may be of interest to record that the cost of the work averages some 4*s*. per acre; while this alone appears to render the scheme a sound investment from the national point of view, its value should not be judged solely on the increase in production on the fields actually sampled. The demonstration to farmers that the fields they are ploughing have high wireworm populations and that the latter can be countered by measures of good cultivation and the correct selection of the crop has a widespread influence, and if the consequent increase in production could be assessed, it would probably far exceed that obtained in the few thousand fields actually sampled. The greatest need at present perhaps

is for a simple and speedy method of separating wireworms from soil on a big scale, so as to avoid the cost and labour of hand sorting. If this could be devised, the cost of the work and the area that could be handled would be greatly extended.

These notes show how the wireworm problem has been dealt with as an emergency measure: it is of course regrettable that the practical applications of the work should have had to be put into force before the preliminary investigations could be satisfactorily completed, but as other papers will show, concentration on advisory work does not preclude the accumulation of new knowledge or even the encouragement of straightforward research, and there can be little doubt that by the end of the war agricultural entomologists will be better equipped to deal with the wireworm problem than they ever were before.

(*Received* 10 *October* 1941)

WIREWORMS AND CROP PRODUCTION

By S. G. JARY, B.A., Dip. Agric. (Cantab.)
South-Eastern Agricultural College, Wye, Kent

The conversion of a large acreage of grassland to arable during the past 2 years has again brought wireworms into prominence as did a similar ploughing-up campaign in 1916–18. Despite the fact that wireworms have long been considered as pests of major importance, the development of direct control measures applicable to agricultural as distinct from horticultural crops has made little advance during the present century, a period in which the science of pest control has made many notable contributions to crop production. The reason is partly to be found in the lack of knowledge of the feeding habits and general ecology of wireworms, due to difficulty in breeding them and maintaining close observation and also to the absence of knowledge of their distribution over large areas. Moreover, the life cycle of the species of economic importance is about 4 years and the aspiring young entomologist has not been tempted to take up the problems involved in the expectation of rapid results. Thus, at the commencement of the present ploughing-up campaign in 1939, there was little concrete advice which could be given to farmers to help in avoiding the almost certain losses. Circumstances imposed upon Advisory Entomologists the necessity of giving advice of immediate application value; there was no time to commence a comprehensive study of new methods of control. Only two alternatives therefore seemed to exist: (1) to apply such direct control measures as were known; in practice these were negligible from the agricultural point of view; (2) to ameliorate the extent of damage by such means as were available, i.e. accepting wireworms as a necessary evil and concentrating upon those methods best calculated to produce crops in spite of the attack.

Wireworm populations in grassland. Among the many questions concerning wireworms, on which information would have been welcome, that of the size of populations in various fields was obviously outstanding, since there was reason to suppose that great variation in numbers might exist from district to district or even from field to field. This was a reasonable supposition, because the same crops on ploughed-up grassland were by no means all equally attacked. Although other factors, particularly those of a cultural nature, might have accounted for these observed differences in the intensity of crop injury, such factors were even more difficult to assess than the wireworm populations, so that clearly a knowledge of the latter was desirable. Some estimates of wireworm population had previously been made (Roebuck, 1924) and later, Ladell (1938), investigating soil insecticides, showed that by taking random soil samples of known size and extracting wireworms from them, it was possible to obtain a knowledge of the population of any given area within ascertainable limits. Clearly, if sampling of this kind could be applied on a large scale, a knowledge of the populations thus obtained would enable valuable advice to be given to farmers on the most suitable crops for the sampled fields. This seemed a possible development which might greatly assist in the production of crops, and a number of Advisory Entomologists at once applied the technique on a field scale. In 1938–9 encouraging results were obtained, and in 1939–40 sampling on a much larger scale was carried out all over the

country, based on a scheme agreed by the Advisory Entomologists in co-operation with the Plant Pathological Laboratory of the Ministry of Agriculture and Fisheries, and the Statistical Department of Rothamsted Experimental Station. After preliminary work with samples of various sizes, it was agreed to take twenty samples of 4 in. diam. from each field; in practice a given field was divided into ten roughly equal plots and a pair of samples taken at random in each plot. Wireworms were usually extracted from the samples by hand sorting, but flotation methods, similar to those of Ladell, were also tried. From the ten pairs of figures thus obtained, tables were compiled at Rothamsted showing the limits of population for any observed density of infestation. Throughout the country, wireworm sampling is now based upon this method, samples as a rule being taken back to the laboratory for sorting, but, in a few instances, examination in the field is possible. The results obtained up to the end of the spring of 1940, involving several hundred fields, showed that wireworm populations do, in fact, vary from field to field in very marked fashion, but that for all practical purposes the population of any given field is distributed fairly evenly, and rarely in such a way that it is desirable to crop one part differently from another. The highest populations found by sampling in this way exceed 3 million per acre, fields of over 1 million being common. There is also evidence that populations are highest in the south-east and decrease toward the north-west of the country. In 1940–1 a still more extended programme of sampling was carried out, covering several thousand fields, so that much more information has now become available on which to base advice about the cropping of fields of known populations.

The suitability of different crops for fields of known population. Evidence has accumulated from general observations dating back at least to 1916–18, that crops differ markedly in their susceptibility to injury by wireworms, though precise relationships have never hitherto been established, since the populations of fields under observation were not then known. It is now possible to make a much more accurate estimate of this relative susceptibility. Some observations on the point were made in 1938–9, but in 1939–40 plant counts were carried out on a large number of fields of known population and crop yields eventually obtained from many of the same fields. Insufficient numerical data were obtained for all the common farm crops, but some, particularly oats, were well covered in this respect, and in any case it was possible to obtain a much clearer idea about the susceptibility of various crops than had been possible before. Periodically the Conference of Advisory Entomologists has reviewed the position and revised the cropping recommendations as more knowledge has become available.

Consideration of crop susceptibility raises another important point, namely, the period over which the field has been in cultivation. It is known that wireworms live about 4 years, and for all practical purposes egg laying is probably confined to grassland; at any rate, if eggs are laid in arable land, comparatively few wireworms survive beyond the early instars, so that, in any grass field which has been down for more than 4 years, one would expect to find larvae of all ages from 1 to 4. Conversely, when a field is broken up, the wireworm population would be expected to diminish each year, as fully grown wireworms pupate in the summer and mortality, incidental to the processes of cultivation, exposure to birds, etc., occurs. What is the extent of the diminution in population each year, and how do conditions in newly ploughed grassland, as distinct from fields which have been ploughed over a longer period, affect the degree of injury likely to be caused by a given population?

Though the maximum population is present in the first year a field is broken, the turf layer, in which most of the wireworms are congregated, remains relatively undecomposed in most soils for a sufficiently long period to enable crops to become established before the peak of the attack occurs, but, in the second and subsequent years, this factor is absent and the population, though smaller, will necessarily concentrate upon the crop plants. It has been found desirable, therefore, to modify cropping advice according to the period for which a field has been in cultivation, a given population in the second year being regarded as potentially more dangerous than a similar population in the first year. Also, by reason of the fact that there is an inherent error in the estimation of populations and because many other factors, such as the nature of the soil, management, etc., certainly have an influence on crop performance, it is thought desirable to base advice upon population groups rather than strictly upon the observed population. The following recommendations are based upon such groups. Though many other crops than those enumerated are grown upon newly broken grassland, they constitute a relatively small acreage, by far the greatest area being devoted to the crops mentioned below.

(1) *Newly broken fields.* It is assumed here that the crop will be sown as soon as possible after the actual ploughing, so that the turf is still only slightly decomposed at that time. The greater the interval between breaking and sowing, the more nearly the conditions approach those of a field in its second year from breaking and the greater is the probability of injury.

Population: wireworms per acre:

>1,000,000. Only flax (linseed), beans and peas stand a reasonable chance of success. These are the most resistant of the common agricultural crops, and in practice suffer little significant damage from wireworms.

601,000–1,000,000. Under good fertility and cultural conditions many crops may be grown, but there is considerable risk of injury except to peas, beans and flax. Wheat, unless early sown, may be seriously thinned. Little is known about winter oats, but spring oats, perhaps the most popular cereal crop following grass, often suffers considerably. Most of the plant counts and crop-yield data (referred to above) were obtained from spring oats, and there is a clear indication that the yield suffers to the extent of rather more than 1 cwt. of grain per acre with each additional 100,000 wireworms. Barley, perhaps because it can be sown later than oats in many seasons, shows evidence of being the safest of the cereals, while rye is useful on some very light soils. Silage crops, e.g. oats mixed with tares, peas or beans, stand well, as might be expected, but the cereal part of the mixture may be badly attacked.

301,000–600,000. In this range cereals are reasonably safe, though some degree of injury is common. Sugar beet and various *Brassica* crops, and potatoes (if lifted early) may be grown.

<300,000. This population range is considered safe for general agricultural crops.

(2) *Fields in second or subsequent years from original ploughing. >600,000.* Few crops except peas, beans and flax are safe, and these may sustain some slight injury. Cereals can be grown only if given the best of treatment.

200,000–600,000. Cereal crops may suffer considerable injury, especially near the higher limit. In all cases the necessity for sound cultivation and attention to fertility conditions is emphasized. Many *Brassica* crops are likely to be successful, though there is relatively

little information about them and silage crops may also be grown. Sugar beet and potatoes should be avoided.

<200,000. Cereals, sugar beet and potatoes are recommended in this range.

Populations in the second year from the original ploughing. Until the autumn of 1940, comparatively little information existed about populations on such fields. It might be expected that a decrease in numbers would be found as compared with the first year, for reasons outlined above, and in most fields, but by no means all, this decrease is found. In some fields the population is of the same order as in the first year, and the question arises whether this is due to sampling error or other factors. As Dr G. Salt has shown, the population as determined by the Advisory Entomologists is not the whole population, the majority of wireworms in the first and second instars and some of those in the third being missed, owing to their small size; in fact, the 'advisory' population is about 15–20% of the total. It is, however, a fair indication of that part of the population, i.e. the larger wireworms, calculated to cause the crop injury. Assuming that these smaller wireworms are not found in the first year and that they have not all perished in the interval, most of the survivors will be large enough to be detected in the second year and will compensate for, or possibly outweigh the loss due to, pupation and other causes. However, there is a high natural mortality among these smaller larvae in grassland, in any case; if there were not, we should find a tendency in permanent grassland toward a continuously increasing population, and this does not seem to occur. It might be expected that the larval mortality would be increased under conditions of cultivation, and, as already indicated, this is often apparently the case, but, where populations as determined by advisory methods remain at least constant over 2 years, while the field is under cultivation, it seems likely that many of the early instar larvae survive. It is not impossible that, under some conditions, the factors responsible for larval mortality in grassland, e.g. predators, larval diseases, etc., are more adversely affected by cultivation than are the wireworms themselves, and this may account for the apparent survival of large numbers of the small larvae in some instances.

The reliability of the population figure arrived at by sampling. This is clearly a matter with which the statisticians are primarily concerned, even allowing for the fact that the advisers do not determine the total population. Most of the Advisory Entomologists feel confident that the population obtained by sampling unploughed or newly ploughed grassland is a reliable guide to crop performance but, when fields have been fallowed or cultivated in some other way before being sampled, the physical condition of the soil is such that the accuracy of the sampling may be less than in unploughed grass. Again, in stubbles, where the irregular distribution of weeds may lead to an aggregation of wireworms in an unnatural manner, sampling results may be erratic. As more data are obtained on these points, it will be possible to say whether or not the accuracy of sampling is sufficiently great for practical purposes, but the present indications are that no very great additional error is involved.

The economics of sampling. The method of sampling in use is slow and laborious and, even with the largest practicable staff, only a small proportion of the land being broken can be dealt with. Therefore, how far is it worth while to proceed with sampling on a relatively small acreage? From data obtained it appears that an average cost for sampling is about 4*s.* 0*d.* per acre; the present value of wheat, assuming a crop of 1 ton per acre, is £14. 10*s.* 0*d.* per acre. In the south-east of England, almost exactly 50% of the fields sampled show a

population of over 600,000 wireworms per acre, i.e. an infestation calculated to result in at least a poor crop. If therefore cropping is planned without a knowledge of the wireworm population, there is about an equal chance that a poor crop will result, so that obviously there is a great deal to be gained by adjusting the crop to the population, putting the resistant crops on the highly infested fields and vice versa.

Populations and soil or other characters. The information obtained about wireworm populations and their distribution would be much more valuable if there were evidence of the existence of districts of higher and lower infestations, associated with soil type or some other factor capable of more rapid determination than the population itself. There is at present little indication that any grouping can be done effectively on these lines, since both light and heavy soils show all ranges of population where they have been farmed under ordinary conditions. Contrary to previous belief, however, it is found that derelict land, e.g. bracken-covered parkland, normally has a very low population and that many of the very best pastures are heavily infested. An exception appears to occur in the case of derelict 'black-fen' land, where populations are commonly high.

The effect of the 'farmer' factor in crop production. It has been said that 'a farmer may be judged by the weeds he grows'; it is almost, though not quite literally, true to say that he can be judged by the amount of wireworm damage to his crops; in other words, the population effect on the crop is largely masked by the treatment which the crop receives at the hand of the farmer. The management factor is all important; the level of cultivation and fertility, the efficiency of the drainage, the use of good seed and sufficient of it, are all of vital importance when the final yield is the criterion. Wireworm damage is serious because it is perhaps the most widespread *single* factor in crop destruction, but, when all else is in favour of the crop, a very large wireworm population can be supported with surprisingly little effect upon the final yield. Thus excellent crops of wheat and oats have been seen on fields where the population was known to exceed 1 million per acre in the first year and of wheat with a similar population in the second year, but these are exceptional occurrences and the work of farmers of acknowledged ability. The great majority of grassland which has been ploughed in the south-east counties, as chemical analysis shows, is normally very deficient in phosphates and often in lime, a state of affairs which reflects the general low level of agriculture practised upon it, and could hardly be expected to give such results unless very generously treated.

Benefits incidentally conferred by wireworm sampling. In many parts of the country, co-operation between advisory departments enables the samples taken for estimation of the wireworm population to be used also for chemical analysis, so that in practice the farmer can also be informed of the fertilizer and lime requirement of his fields at the same time as the wireworm population. In the majority of instances, these fields would not otherwise be sampled for chemical analysis. Again, in the course of crop examination in the spring and summer, on sampled fields, many instances of troubles erroneously attributed to wireworms are encountered, resulting in a very real spread of general educational influence among farmers, many of whom have had a very limited experience of arable cropping. Injury to spring oats by frit-fly is an outstanding example of this, while leather-jacket control, often just as important as wireworm, on newly ploughed grassland, has been a matter of primary importance in the spring months. Very widespread and severe cases of manganese deficiency on some soils also have been noted and reported to the chemist

concerned. In brief, well-trained and experienced workers, having greater facilities than ever before to make contact with a large number of farmers, have the opportunity to contribute to crop production in many ways other than their immediate work of sampling fields. The general attitude of many farmers is that they have no time to ask for information, they much prefer to be told what they should do to produce the best results.

REFERENCES

LADELL, W. R. S. (1938). Field experiments on the control of wireworms. *Ann. appl. Biol.* **25**, 341.
ROEBUCK, A. (1924). Destruction of wireworms. *J. Minist. Agric.* **30**, 1047.

(*Received* 10 *October* 1941)

STATISTICAL PROBLEMS IN FIELD SAMPLING FOR WIREWORMS

By F. YATES, Sc.D. and D. J. FINNEY, M.A.
Rothamsted Experimental Station, Harpenden, Herts

(With 3 Text-figures)

The results obtained from the Wireworm Survey in the harvest year of 1939–40, the first in which sampling technique was used extensively for assessing wireworm populations, have been described (Anon, 1941; Finney, 1941). During the 1940–1 harvest year a very much larger number of fields, both grass and arable, was sampled, and these figures are the basis of the present paper. They enable the earlier brief discussion of sampling errors to be amplified and the limits of error to which any sampling is subject to be estimated with much greater confidence. The geographical distribution of populations is also examined and compared for arable and grass fields. It is, however, not yet possible to discuss the effects of wireworms on the 1941 harvest.

The problem

The determination of the degree of infestation of fields with wireworms is of use from the immediately practical viewpoint of deciding what crops it is safe to grow on these fields, and whether it is advisable to plough up grassland for arable cropping. It is also of value in the more general study of the distribution of wireworms throughout the country, and of their variation from season to season. If wireworm counts are to be of practical use it is necessary, firstly, that the actual populations shall be determined with a satisfactory degree of accuracy; secondly, that the methods of sampling adopted shall be those which require a minimal amount of time and effort; and thirdly, that the correlation of the determinations with subsequent wireworm damage shall be ascertained. These problems are statistical in nature, and have been solved in general terms in recent years. Their application to wireworm sampling is an illustration of the methods which can be followed in many other fields of inquiry of a similar type.

General principles of sampling

The practical object in taking a sample count from a field is to determine the mean wireworm population per acre of that field. If the observer is satisfied that the mean so determined is sufficiently accurate for his purposes, he need not concern himself with the calculation of sampling errors, or even consider whether the method he is using enables them to be calculated. When a new method of sampling is being developed, however, it is important to be able to estimate its precision, and this can only be done by the calculation of sampling errors. It is therefore necessary in the first instance to choose methods of sampling in which the calculation of sampling errors is possible. When an estimate of the accuracy attained with a given intensity of sampling has been made, the intensity which will be required in subsequent work can be decided. There will then be no need to con-

tinue with the calculation of sampling errors on all fields, though it will, in general, be desirable to perform the calculation for a few fields as a routine measure in order to see that conditions are not changing.

The estimation of the sampling errors associated with any given method of sampling is very simple in principle: the estimate is based on the discrepancies which occur between the components of the sample taken from the same part of the field. Certain conditions must, however, be observed when taking the sample if these discrepancies are to give a valid estimate of the sampling error. The main condition is that the components (or *sampling units*) must be taken at random, either from the whole field or in equal numbers from equal areas of the field. If, for example, twenty sampling units are taken at random from a 10-acre field, the sample so obtained will not be as accurate as if two sampling units are taken at random from each of the 10 separate acres of the field, since the second procedure will eliminate from the sampling error any real variation between the different acres. By the same line of reasoning, the aggregate of 20 sampling units, one taken at random from each of the 20 half-acres, will in general be still more accurate. Unfortunately, it is impossible to determine with certainty the accuracy of this last procedure, since there is only one sampling unit from each division of the field. Whenever exact estimates of the sampling errors are required a certain amount of accuracy must be sacrificed in order to enable them to be calculated.

When the procedure is finally standardized it will often be found more convenient, and will probably be a little more accurate, to adopt the routine of taking one sampling unit from each half-acre rather than two from each acre, if the latter procedure has been proved to be sufficiently accurate. There is thus a conflict between the conditions that must be fulfilled if an exact estimate of the sampling error is required, and the conditions which give maximum accuracy. This has from time to time caused a certain amount of misunderstanding. Further simplification of the sampling procedure is possible if we relax the condition that the samples shall be taken at random. In doing this there is always a slight risk that bias may be introduced; if we take samples at the centre of each of the 20 half-acres of the field we shall not obtain any samples close to the edges of the field, and if the population density were habitually lower on the edges, there would be a tendency to overestimate the average population.

In each particular problem it is necessary to decide whether such biases are likely to arise in the material which is being handled and whether, even if they do arise, they are of any consequence, having regard to the purposes for which the sampling determinations are required. In the case of wireworm surveys, a slight bias is not of importance, since it merely means that all the estimated populations are slightly higher or lower than they should be. In other types of problem, however, complete elimination of bias may be of the greatest importance.

History of wireworm sampling

Though field sampling for the estimation of wireworm populations has been in use for a number of years, there was little available information on the magnitude of the sampling errors or the most efficient size of sampling unit. Sampling carried out at Rothamsted Experimental Station by Ladell (1938) suggested that sampling units 6 in. square were more efficient, volume for volume, than 9 in. square units. Jones (1937), in America,

experimented with different sizes of sampling unit, but only reached the obvious conclusion that, for a given number of sampling units, more precise estimates are given by larger units. Clearly the practical comparison is that of a large number of small units with a few large ones; when Jones's results are examined in this light, they are seen to be in good agreement with the present investigation.

The first recommendation for the present survey was that the sampling unit should be a 6 in. square of soil (with the turf on unploughed fields) taken to a depth of 6–9 in. so as to obtain most of the wireworms. For a field of average size twenty of these sampling units were to be taken, two being located at random in each one-tenth of the field. The comparison of these pairs was used to estimate the sampling errors. About 100 fields were sampled in the 1938–9 harvest year, mostly by 6 in. squares, but a few with sampling units of other sizes. As a result of the statistical examination of the sampling errors of these fields, and from general considerations, it appeared that, for a given volume of soil examined, a sampling unit smaller than the 6 in. square was likely to give more precise estimates. Accordingly it was decided that in the 1939–40 work a cylindrical core of 4 in. diam. should be the standard sampling unit. If the same number of sampling units per field is taken, the volume of soil to be examined in 4 in. cylindrical cores is little more than one-third that of the 6 in. squares. Sample counts are easily transformed to an acreage basis when it is remembered that the area of one 4 in. diam. sampling unit is almost exactly 1/500,000 acre.

Examination of the wireworm counts obtained showed sampling errors of the order anticipated, and the same sampling procedure was therefore continued in the 1940–1 season. The only modifications introduced were that, whereas in the previous years very few larvae of less than 8 mm. were counted, it was now recommended that all exceeding 5 mm. should be counted, and also that the counts should be limited to *Agriotes* spp. Previously occasional larvae of other species had been included, but their numbers were very small by comparison with those of *Agriotes obscurus*.

Sampling errors

The discussion (Finney, 1941) of sampling errors was based on records from 473 fields sampled in the 1939–40 harvest year, of which only 309 provided information on the variability of counts in 4 in. cores. The more numerous data obtained in 1940–1 have now been analysed, and are in excellent agreement with the earlier figures. In addition to old grass fields sampled before or shortly after ploughing, a number of fields which had been ploughed a year earlier and cropped in 1940 were sampled, largely because they had suffered crop failures. These 'arable' fields were kept apart from the 'grass' fields in the statistical examination of the data.

If individual wireworms in a field were distributed randomly, and entirely independently of each other—that is to say, if there were no tendency even for very localized patchiness—the numbers found in different sampling units would follow a Poisson distribution. In this the standard error of the number per sampling unit is equal to the square root of the mean number per sample. Unequal oviposition, variation in food supply, and variation in suitability of environment are all likely to introduce a certain amount of patchiness, and, therefore, the sample counts are almost always more variable than the Poisson law predicts.

In order to obtain an empirical law, the standard error of sampling for each field of the 1940–1 Survey which was sampled by 4 in. cores was expressed as a percentage of the

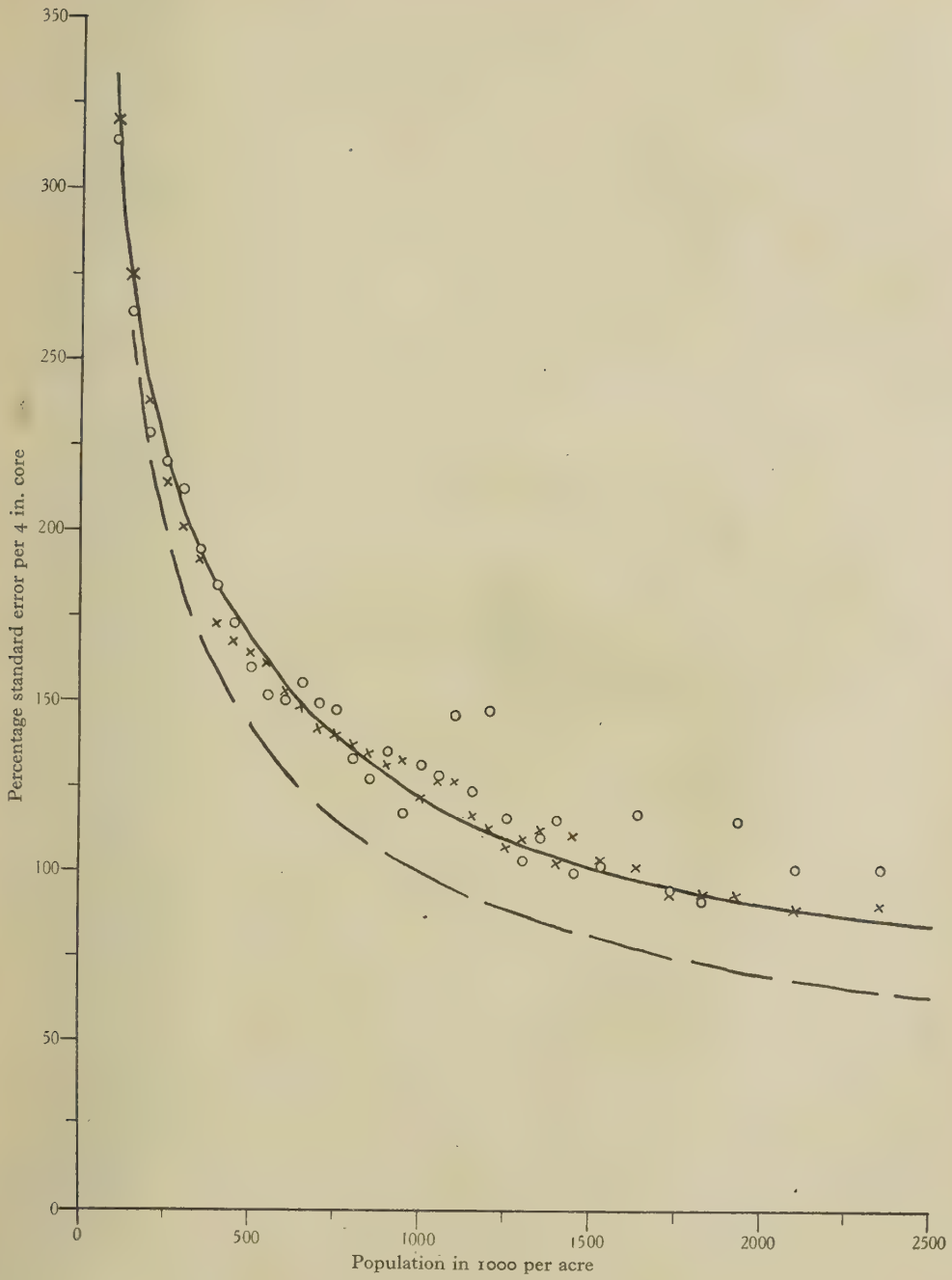

Fig. 1. Standard errors per unit core of 4 in. diam. (wireworm survey 1940–1).

×: means for 2272 fields grass in 1940.
o: means for 525 fields arable in 1940.
—— fitted to data from grass fields.
- - - - Poisson distribution.

mean population of that field, and these percentages were plotted against the mean population. Fig. 1 shows the mean percentage standard error at different levels of population for both grass and arable fields. An empirical curve has been drawn for the grass fields. This curve agrees very closely with that drawn for 309 fields in the previous year, being almost indistinguishable on the scale used.

It was thought that, for any given population, arable fields might have greater sampling errors than grass, on account of the aggregation of wireworms when feeding along the lines of drilling or round individual plants. For populations below 1 million per acre there is little sign of this effect, but above that level the few fields which have been sampled give some support to the opinion. Further data are required before forming any definite conclusions, but probably allowance should be made for greater variability under arable conditions at the higher levels of population.

Some workers considered that variations in wireworm population in different parts of the same field might be of importance. This point was discussed by Finney (1941), who found that the variability between the means of the ten pairs of samples per field is much the same as that between members of the same pair. This analysis has not been made in such detail for the 1940–1 records, but again it is apparent that, for the vast majority of fields, there is little evidence of any marked variability between different parts of the same field (as distinct from the small scale concentrations of larvae discussed above). Undoubtedly there are exceptions, and when such are found further sampling of the field may be desirable if the danger areas are to be satisfactorily defined. It is often observed that crop failures ascribed to wireworm are of a patchy nature, but this may be more frequently a secondary effect of the lessened powers of plant recovery on areas of poor soil fertility than a primary effect of uneven wireworm infestation.

Probable limits of error

Standard errors obtained from the figure may be used to prepare tables showing the extent to which sample estimates of populations may be expected to err from the true values; the first half of Table 1 shows the case of twenty sampling units per field. Thus fields having 400,000 larvae per acre will in one case out of eight give estimates too low by 140,000 or more, and in one case out of eight estimates too high by 140,000 or more. This accuracy is, of course, proportional to the square root of the number of samples.

From Table 1 we may deduce (by interpolation) that the population which has 300,000 per acre as its lower limit is 449,000 per acre. That is to say, if an estimate is found to be 300,000 per acre, it can be asserted with reasonable confidence that the true population is less than 449,000 per acre, and similarly that it is more than 204,000 per acre. The second half of Table 1 shows these limits, which are technically called *fiducial limits*. A table of fiducial limits is required when assessing the degree of confidence that can be placed in any particular estimate arrived at from a sample count. Similar tables can be drawn up for samples containing different numbers of sampling units and for probability levels other than the 1 in 8 used here; e.g. a similar table has been prepared for the 6 in. square sampling unit used in the Midland Agricultural Advisory Province.

The two halves of Table 1 are very nearly the same as the corresponding portions of Tables 3 and 4 of the earlier paper (Finney, 1941). This is a necessary consequence of the close agreement found between the standard errors of sampling in 1939–40 and 1940–1.

TABLE 1. *Distribution and probable limits of error of sample estimates of populations of grass fields sampled by twenty 4 in. cores*

(1000 per acre)

True population	One-eighth of sample estimates: Less than	One-eighth of sample estimates: Greater than	Estimated population	Population which, in one-eighth of cases, would give an estimate: Not less than that observed	Population which, in one-eighth of cases, would give an estimate: Not greater than that observed
50	9	91	50	—	118
100	38	162	100	56	193
150	71	229	150	92	261
200	105	295	200	128	325
250	142	358	250	166	388
300	180	420	300	204	449
400	260	540	400	284	567
500	343	657	500	367	686
600	428	772	600	451	804
800	597	1003	800	624	1040
1000	766	1234	1000	797	1277
1500	1189	1811	1500	1230	1868
2000	1612	2388	2000	1664	2459

A density of 100,000 per acre corresponds to 4 larvae per 20 samples.

RELATIVE EFFICIENCY OF 4 IN. CORES AND 6 IN. SQUARES

The routine testing of fields for wireworms is organized by the Advisory Entomologists of the thirteen Agricultural Provinces of England and Wales. The Midland Province, in 1940–1, provided an extensive series of results using 6 in. square sampling units, and a fresh determination of the sampling errors associated with this sampling unit was made. If it be assumed that the labour of separating wireworms is directly proportional to the amount of soil involved, it is possible to assess the relative efficiency of the two sizes of unit. The relative cost of using small sampling units may be slightly increased by the larger number of units to be dug, but this is probably compensated by a psychological advantage in examining a given volume of soil in small units rather than large. Table 2 shows the standard error per sample for 4 in. cores and the relative values of equal volumes of soil obtained by the two methods of sampling at various levels of population.

TABLE 2. *Comparison of efficiency of sampling units per unit volume of soil examined*

Population 1000 per acre	Standard error per 4 in. core 1000 per acre	Percentage relative efficiency of 6 in. squares
25	112	92
50	161	75
75	203	71
100	241	69
200	368	67
300	466	65
400	544	63
500	610	62
1000	910	59
1500	1210	58
2000	1510	58

The volume of one 6 in. square soil sample is $\times 2{\cdot}86$ that of one 4 in. core.

For very low populations the precision of the estimate of population obtained from a given volume of soil is the same whatever the size of sampling unit employed, but the efficiency of the larger unit decreases rapidly with increasing population. In fields containing over 1 million wireworms per acre about 70% more soil is required to obtain a given precision if 6 in. squares are used instead of 4 in. cores; thus twelve squares would be required to replace twenty cores instead of only the seven required to give the same volume. On general grounds it seems likely that a further reduction of the size of the unit sample, e.g. to a 3 in. or 2 in. core, would be still more efficient, but difficulties would be encountered in driving so small a core into the rougher pastures. It is hoped that this point may be investigated during the 1941–2 harvest year.

Leatherjackets

In some Provinces counts of leatherjackets were also made during the 1940–1 sampling, the same set of soil samples being used. The populations found were generally low by comparison with wireworms, and showed similar variability within the field. The few fields with more than 500,000 per acre suggested that at these higher levels the sampling errors became greater than the corresponding wireworm figures and that more cases occurred of exceptional samples, containing fifteen or more larvae. A table of the same type as Table 1 has been prepared and both parts show limits a little more widely spaced than for wireworms. There were, however, too few fields with populations above 500,000 per acre to give much information in this range.

The sampling technique is likely to be less satisfactory for estimating the danger to crops from leatherjackets, unless many more samples per field are taken. Effective discrimination between populations of 50,000 and 100,000 per acre is not possible with only twenty sampling units per field, yet differences of this order are generally considered to have important bearings on leatherjacket damage.

Geographical distribution of populations

When the estimates of population for the fields sampled in 1939–40 were examined, very marked differences between the thirteen Agricultural Provinces were found. Such differences might, in part, be due to variations between teams of observers in the technique of counting the larvae in the samples. The general practice was to transport samples to the laboratory and there to separate the wireworms by hand. In the Western Province, and occasionally also in the South-western, a flotation process was used for separating the larvae. In the Yorkshire and North-western Provinces samples were examined on the field, as was also the case for arable fields in the Northern Province and, under favourable conditions, some fields in North Wales. Again, small larvae will be discovered less easily in heavy than in light soils, and personal factors influencing observational power will contribute to Provincial differences.

Nevertheless the geographical trends in mean populations appeared too regular to be entirely the chance results of any or all of these causes. Fig. 2 shows a very clear gradient from south-east to north-west. Thus in the three provinces of the south-east of the country 86% of the fields had populations in excess of 300,000 per acre, whereas in the four most northerly provinces only 18% exceeded this figure.

The much greater number of fields sampled in 1940–1 enabled the geographical distribution obtained in this season to be examined in greater detail. One difficulty arose. In the previous year Advisory Entomologists had little knowledge of the distribution of wireworms in their areas, and the selection of fields was likely to be free from bias. In some instances this selection was scarcely representative of the whole Province, occasionally being

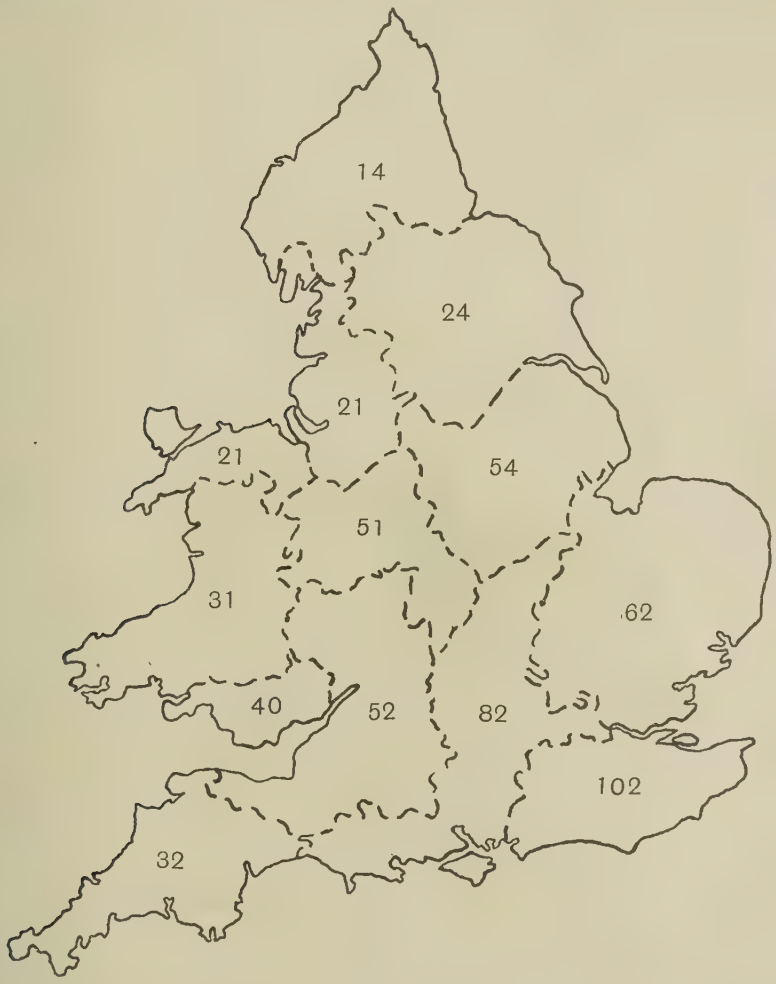

Fig. 2. Mean populations of Provinces, 1939–40 (in 10,000 per acre).

almost confined to a single county, but there was no reason to suppose that districts of especially high or low infestation were chosen. In 1940–1, however, the position was very different. From the information given by the earlier samplings some Advisers believed that they knew of regions of high and of low infestation within their Provinces. Since the requirements of a pure research programme were necessarily of secondary importance to those of efficient advisory work, it was natural that efforts should be concentrated on the

heavily infested areas, in which sampling and subsequent advice on cropping might be expected to give the best returns. In many Provinces, also, requests for sampling were received from farmers who had suffered failures attributable to wireworms in 1940. This latter effect will, of course, cause a biased selection primarily for the arable fields, but, if there is any correlation of populations between fields of the same farm, the representative nature of the grass fields may also be affected. Though it is not yet possible to discuss this correlation in detail, evidence so far examined shows that a substantial association between populations of fields on a single farm frequently occurs.

Enquiries which were made as to the procedure actually followed in each of the Provinces, indicate that only in the Eastern, South-western, and Yorkshire Provinces is there likely to have been much bias in the selection of grass fields. In these three there were conscious attempts to sample more intensively in regions of high population.

A population gradient similar to that of the previous year is noticeable in the results shown in Table 3, though the populations in the more heavily infested Provinces are very much reduced. This general reduction in the population level in 1940–1 is the more remarkable in view of the alteration from 8 to 5 mm. in the minimum length of larvae to be recorded. If, in fact, all wireworms of this size group were counted in the second year, they might be expected to form a large proportion of the total. Since increased populations were not found, it seems likely that these smaller larvae were frequently not discovered, and that the threshold of observation for the ordinary methods of sample examination is at least 8 mm.

The arable fields show somewhat different results. In one Province no arable fields were sampled, except for those resampled after the previous year's Survey; these results will not be discussed here. In the remaining twelve Provinces the fields were largely selected because of crop failure in 1940. Though such failures were not necessarily due to wireworms, any such method of selection must produce a bias in the mean population for those fields which carried susceptible crops. The fact that, in spite of any bias introduced in this way, the populations found were generally substantially less than in the grass fields,

TABLE 3. *Mean wireworm populations in the agricultural provinces of England and Wales*

	No. of fields			Populations (1000 per acre)		
		1940–41			1940–41	
Province	1939–40 Grass	Grass	Arable	1939–40 Grass	Grass	Arable
South-eastern	82	290	43	1025	635	520
Southern	33	218	16	825	480	340
Eastern	50	279	63	625	550	300
Midland	42	520	41	540	520	330
Western	16	119	19	520	365	240
West Midland	38	247	67	505	465	475
South Wales	29	384	49	395	350	285
South-western	35	461	97	325	310	325
Mid-Wales	17	339	166	310	220	210
Yorkshire	30	168	62	235	205	275
North Wales	30	395	133	210	295	325
North-western	40	479	0	210	225	—
Northern	31	154	69	135	150	305
All	473	4053	825	520	370	315

agrees with the expectation that the population falls considerably in the first year after ploughing. Differences between Provinces were much less than for grass fields, and apart from high figures in the South-eastern and West Midland Provinces all the mean populations were near 300,000 per acre.

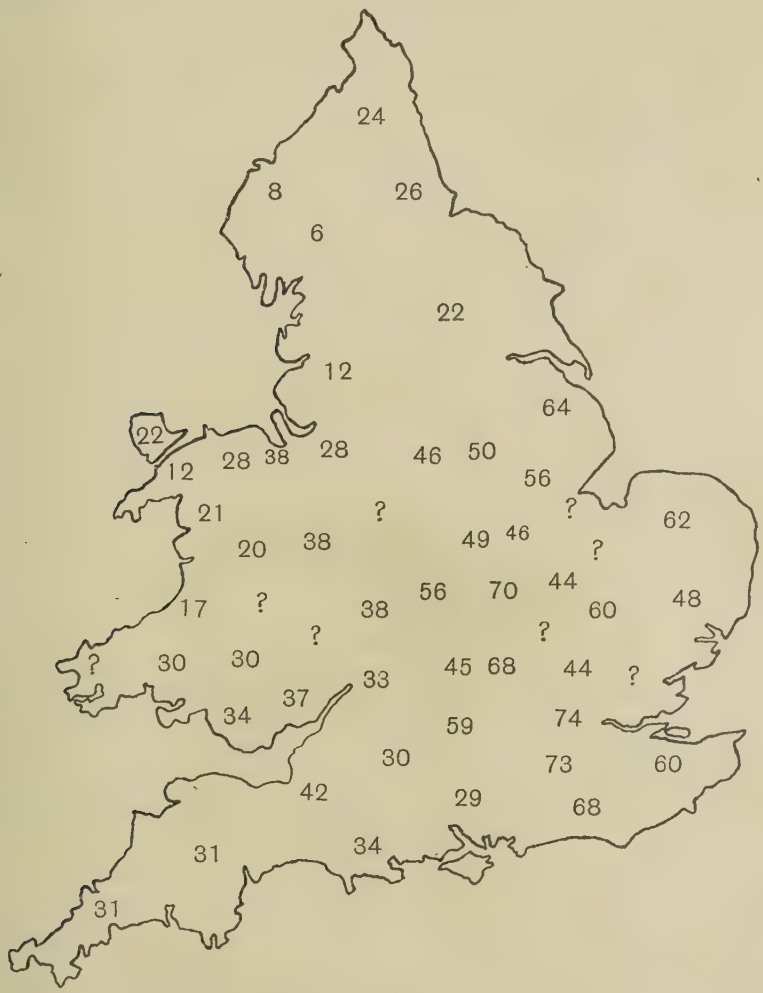

Fig. 3. Mean populations of counties, 1940–1, grass fields only (in 10,000 per acre). Counties with less than ten fields omitted.

With 4000 fields available, it was possible to examine the distribution of populations of grass fields in greater detail. Within each Province a subclassification by County was made: such division is not ideal, as county boundaries can claim no special virtues in the demarcation of areas of high and low infestation. But, unless the population of each field sampled were plotted on a map, it is not easy to see how such areas could be better determined. Mean populations for all Counties in which ten or more fields were sampled in 1940–1 are shown in Fig. 3.

No theoretical reasons can be adduced for the geographical distribution of populations, and only examination of data for a period of years can show whether or not its features are permanent. The results for this second year are in tolerably good agreement with those found a year earlier, though there has been some reduction of the difference between the extremes of the country. Further subdivision of counties may possibly indicate reasons for the occurrence of high and low populations, though classifications which have been tried, e.g. according to soil type or *p*H value, have not proved very helpful.

A further aspect of the mean populations is given by Table 4, showing the proportion of all fields having different levels of infestation. This has been prepared separately for the five Provinces which had the highest populations for the 1940–1 grass fields and for the remainder of the country. The percentage of heavily infested fields in these five Provinces has fallen in the second year of the survey, and is again lower for the arable fields, but in the remainder of the country the three sets of percentages are very similar.

TABLE 4. *Percentage of fields at four levels of infestation*

		Population (1000 per acre)			
		–300	301–600	601–1000	1001–
1939–40: Grass	H	24	24	26	26
	L	72	19	5	4
1940–41: Grass	H	35	31	22	12
	L	67	23	7	3
Arable	H	52	28	14	6
	L	68	20	9	3

H: South-eastern, Southern, Eastern, Midland, and West Midland Provinces.
L: Other Provinces.

EFFECT OF WIREWORMS ON CROPS

No mention has yet been made of larvae smaller than 5 mm. in length. The recent researches of Dr Salt and his colleagues at Cambridge confirm the opinion that these far outnumber the larger ones. Little appears to be known of the feeding habits of the very small wireworms and it is probable that they cause little crop damage. Knowledge of their numbers, however, may be of great value in determining the probable number of large wireworms in subsequent years, and in indicating whether special remedial measures should be taken.

The estimates of the numbers of the larger wireworms have certainly proved a satisfactory index to the yields of oats and wheat, at least in the 1940 harvest, both crops giving much reduced yields in the presence of heavy wireworm infestations. These yields, and also a series of plant counts on the same fields, were discussed by Finney (1941) and nothing can be added until harvest results for 1941 are available.

SUMMARY

A general summary of the principles underlying field sampling, with particular reference to the taking of soil samples for the estimation of wireworm population, has been given. The results of three years of wireworm sampling by the Advisory Entomologists of England and Wales have shown that twenty cylindrical cores per field, each 4 in. diam. and 6–9 in. deep, can be used to give estimates of population with sufficient accuracy. For the total

volume of soil to be examined, this size of sampling unit has been shown to be definitely more efficient than a unit 6 in. square. On general principles, it is to be expected that the smaller the sampling unit used the more efficient it will be, but limitations are imposed by the practical difficulties of working with very small units.

Grass fields sampled in the 1939–40 harvest year showed very much higher populations in the south-east and midland areas than in the north and west. This difference was confirmed by the more numerous data for 1940–1. In spite of a reduction in the lower size limit of the larvae to be counted in the samples, populations in this latter season tended to be lower than in 1939–40. Arable fields sampled in 1940–1 gave populations generally lower than grass fields, and the geographical trend was less noticeable.

REFERENCES

ANON (1941). Wireworms and newly-ploughed grassland. *J. Minist. Agric.* **47**, 251–7.
FINNEY, D. J. (1941). Wireworm populations and their effect on crops. *Ann. appl. Biol.* **28**, 282–95.
JONES, E. W. (1937). Practical field methods of sampling soil for wireworms. *J. agric. Res.* **54**, 123–34.
LADELL, W. R. S. (1938). Field experiments on the control of wireworms. With Appendix: The information supplied by the sampling results, by W. G. Cochran. *Ann. appl. Biol.* **25**, 341–89.

(*Received* 10 *October* 1941)

OBSERVATIONS ON SOME FACTORS INFLUENCING GROWTH IN WIREWORMS OF THE GENUS *AGRIOTES* ESCH.

By A. C. EVANS, Ph.D. and H. C. GOUGH*, Ph.D.
Department of Entomology, Rothamsted Experimental Station, Harpenden

(With 4 Text-figures)

This work originated in experiments set up to study the utilization of organic matter in the soil by wireworms. As the work progressed certain curious facts were noted, e.g. sometimes a wireworm would feed appreciably, yet its weight would remain constant or even decrease; also starving wireworms might remain approximately constant in weight over long periods, whereas at times they might increase in weight without apparent reason. In view of these observations and of the known great variability in the results of experimental work on these insects, experiments were set up to find a technique for rearing wireworms in such a way that the growth of individuals could be studied in detail, with particular attention to changes in weight in relation to food and soil moisture.

The following technique was finally adopted. Wireworms were confined, individually, in glass vessels of varying size according to the size of the wireworm; for small wireworms, 1–20 mg. in weight, vessels 3 × 1 in. were very suitable, but for the larger ones, vessels 5 × 1¼ in. were required. The vessels were one-third filled with soil of a known water content and after the wireworm and food had been introduced the vessels were tightly plugged with cotton-wool. Soil with a moisture content of 45% saturation (Keen & Raczkowski, 1921) was used in general experiments, since it is usually easy to handle and does not stick to the wireworm which can rapidly be cleaned for weighing. The wireworms were weighed weekly on a Joly balance (see Bolas & Melville, 1933); by its use wireworms can be weighed at a rate of sixty per hour. A cone-shaped pan was used instead of the flat type described by Bolas & Melville. The vessels were kept in a heated underground chamber at a temperature of 15° C. except for a fortnight in July when the temperature rose to 18–19° C.: the food was changed weekly. This procedure eliminates the effect of seasonal changes in temperature on growth, but this effect is being studied in investigations now in progress. The use of the Joly balance was suggested to us by Dr A. C. Fabergé, to whom we are indebted for help in its construction.

Growth and moulting

The wireworm spends 4 years in the larval stage, and in order to obtain information rapidly the growth of individuals of various sizes obtained from different sources was studied.

Eggs were obtained from *Agriotes obscurus* in June; these hatched in mid-July, and the mean weight of individuals from several batches was 0·09 mg. By mid-October these wireworms weighed between 0·2 and 0·8 mg., according to the food on which they were reared. No details were obtained on the length of time which these larvae spent feeding. An

* Now of Department of Agriculture, Leeds University.

investigation of the growth of small wireworms weighing 1·1–2·4 mg. was started on 20 Mar. 1941. These grew rapidly at first and fed well on wheat grains or carrot disks for 2 weeks and then ceased to feed although food was present. This fasting phase lasted for 3–4 weeks, during which the wireworms continued to increase in weight (Fig. 1, A, B). During the 30 weeks to 16 Oct. approximately over half the total increase in weight was gained during fasting periods which lasted usually for a total of 14–18 weeks. Moulting occurs during the fasting period usually in the penultimate week: all the wireworms in this batch moulted 3 times. During 30 weeks their weights increased ×6–10. One anomalous individual was observed. Its weight was 1·7 mg. and from 20 Mar. it fed on wheat for 2 weeks, its weight increasing by 0·6 mg., but it has not fed since although given the

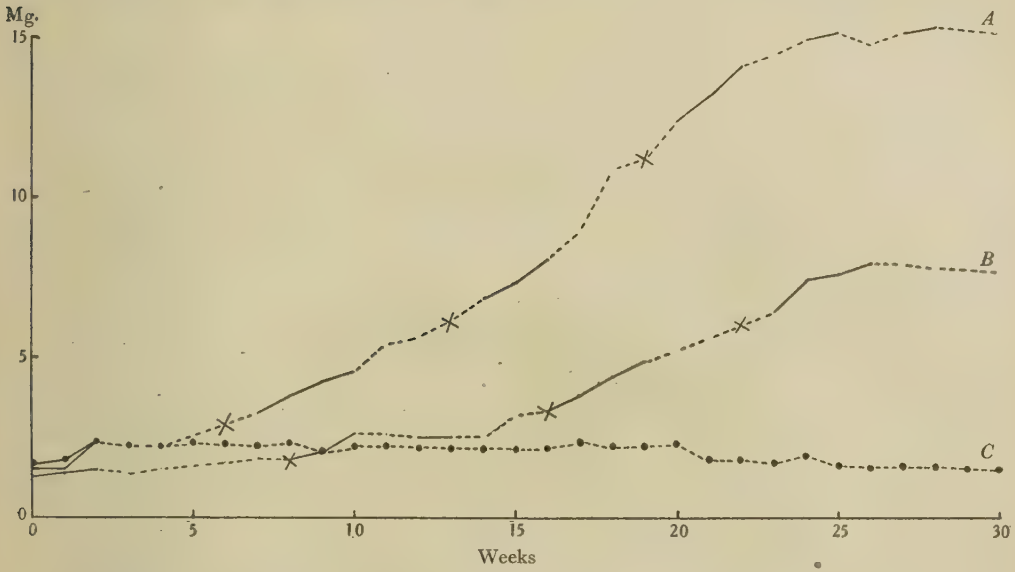

Fig. 1. Growth curves of small wireworms. × Moult. —— Feeding. ---- Fasting.
A, fed on wheat. B, fed on carrot. C, fed on wheat for 23 weeks then on wheat and carrot.

choice of wheat or carrot. After 30 weeks of fasting its weight was 1·5 mg.: moulting was not observed.

Very similar results were obtained on a batch of larger wireworms weighing 5–7 mg., obtained from another source, the only difference being that 30% moulted 3 times and 70% moulted twice. During 30 weeks the wireworms in this batch increased in weight ×3–4.

Wireworms larger than those described above, i.e. 20–25 mg. in weight, do not grow so quickly; during 25 weeks the mean weights of several groups increased in weight by only 10–20%. Analysis of the growth records of over 100 wireworms from April to October 1941 showed that the phenomenon of moulting is more elaborate in larger wireworms than in small ones. At the beginning of the experiment all the wireworms, with a very few exceptions, fed and increased in weight for 2–3 weeks. They then fasted for a period of 5–8 weeks, during which their weight decreased slightly or remained approxi-

mately constant. Then, still fasting, they increased rapidly in weight for a period of 2–3 weeks by an amount varying from 1 to 10 mg. The majority, 80%, increased in weight by 3–6 mg. After this increase in weight the course of events followed by different wireworms varied greatly (Fig. 2). A few, about 10%, remained constant in weight for a week or two, moulted and then fasted for another week before feeding started again. Others decreased

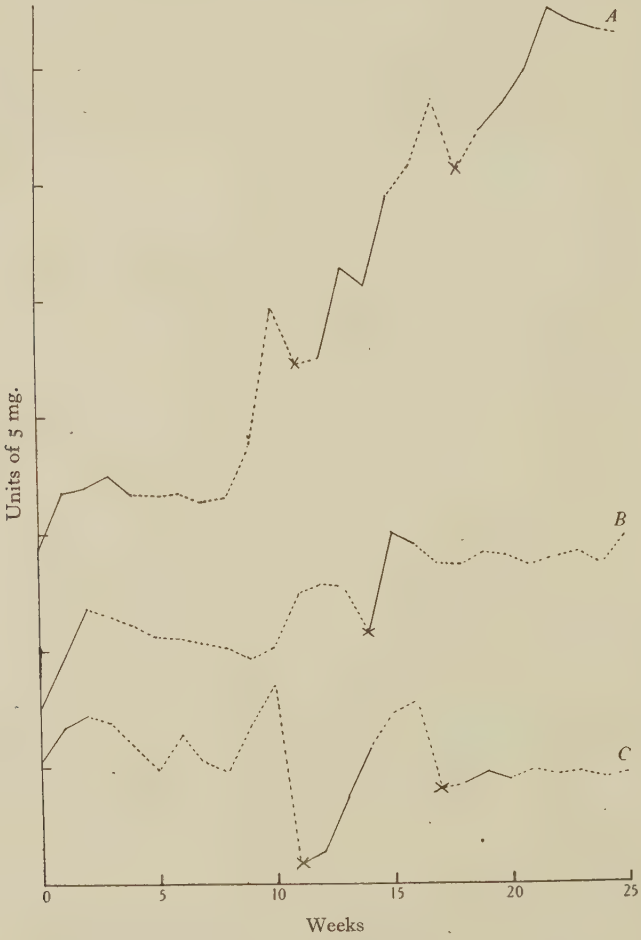

Fig. 2. Growth curves of medium-sized wireworms. × Moult. —— Feeding. ---- Fasting. A, fed on wheat, initial wt. 24·4 mg. B, fed on carrot, initial wt. 22·6 mg. C, fed on potato, initial wt. 25·3 mg.

in weight by variable amounts, 1–7 mg., before moulting, and the moult was generally followed by a week of fasting before feeding started again. There is no relationship between the increase and the decrease in weight before and just after moulting. A large increase in weight may be followed by a small decrease (Fig. 2A), or vice versa (Fig. 2C), or the increase and decrease may be approximately equal (Fig. 2B); other variations are also found. The mean increase in weight was 5 mg. and the mean decrease 2 mg.

Some of these wireworms (60%) moulted once during the experiment, others (39%) twice, and one three times. Those which moulted twice fed for 3 weeks after their first moult and then fasted for another 3 weeks before moulting again. They increased in weight by variable amounts during the feeding period, and during the subsequent 2 weeks of fasting increased by amounts varying from 2 to 8 mg. (mean 4 mg.). They then decreased in weight for a week by amounts varying from 1 to 4 mg. with a mean of 2 mg. and moulted (Fig. 2 A, C). A week's fast usually ensued after moulting before feeding started again.

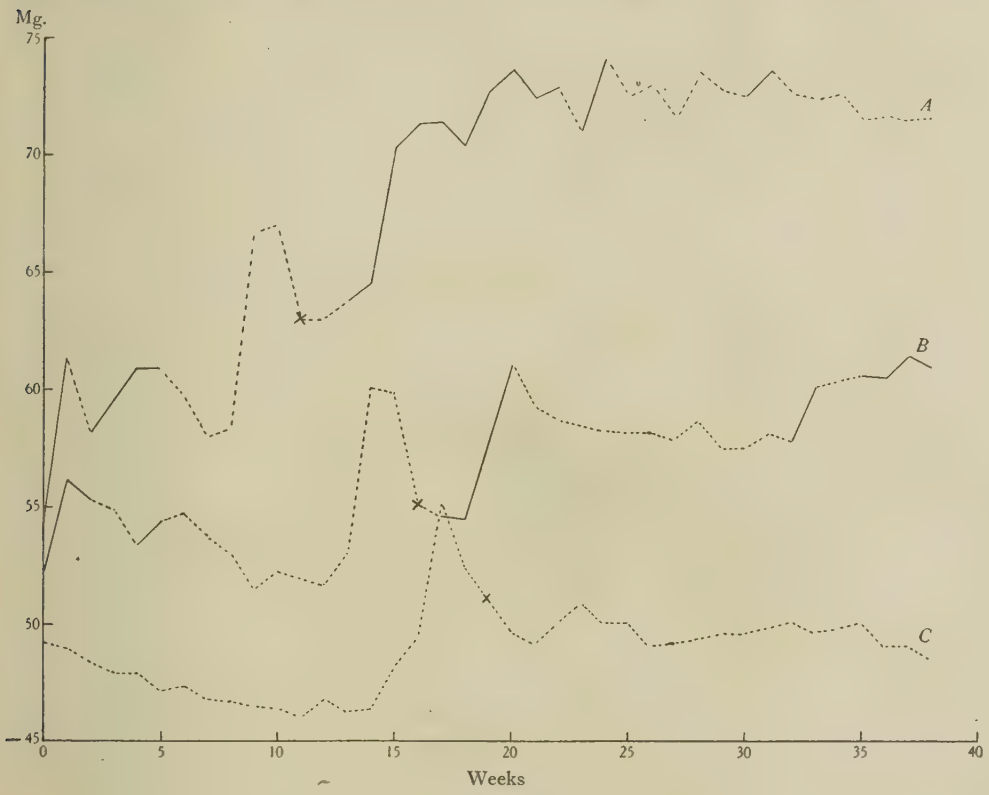

Fig. 3. Growth curves of large wireworms. × Moult. —— Feeding. ---- Fasting. A, fed on wheat. B, fed on carrot. C, no food given.

The wireworms usually fed for 2–4 weeks and then fasted for 2–4 weeks when the experiment was terminated. Those which only moulted once fed for a period of 4–6 weeks after moulting and then fasted until the end of the experiment.

The growth curves of very large wireworms weighing 40–55 mg. were also studied. These moulted once only during the period of observation which so far, October 1941, has lasted 38 weeks. After an initial feeding period of 2–6 weeks, during which they increased in weight, the wireworms fasted for a period of 7–9 weeks, during which they decreased steadily in weight (Fig. 3 A, B). A large increase in weight, varying from 3 to 14 mg. (mean 7 mg.), then occurred during the next 2–3 weeks, this being followed by a

decrease in weight of 3–7 mg. (mean 5 mg.) after which the wireworm moulted. Moulting was followed by a fasting period of 1–3 weeks before feeding restarted. This feeding period lasted for 1–10 weeks, after which the wireworms entered upon a fasting phase which is still being studied. Control wireworms without food also showed similar changes in weight in relation to moulting (Fig. 3 C).

The increase in weight of wireworms preparatory to moulting is an interesting phenomenon. It is due to the absorption of water through the cuticle, since if a wireworm in the fasting phase before moulting has its mouth and anus blocked with wax it is still able to undergo this great increase in weight. This absorption of water preparatory to moulting is also shown by wireworms of the genus *Athous*. The cuticle of insects is generally impervious to water, but a few exceptional cases have been noted by Wigglesworth (1939). Woodworth (1938) studied the absorption of sodium arsenite by wireworms from solutions of varying strength and found that the wireworms (*Limonius canus* Lec.) increased in weight by 60–80 % after submergence in these solutions and in distilled water for 96 hr. He does not give any details as to the size or previous history of his experimental material and does not appear to regard this absorption as occurring at any special time in the life history.

As a result of these varied changes in weight during the first moult of wireworms of 20–25 mg., it might be expected that their percentage dry weight would fluctuate greatly. An experiment was set up to examine this point: 200 wireworms were placed in tubes with carrot as a source of food, weighed at weekly intervals, and classified according to their physiological state in relation to the moulting curve. A sample of twenty individuals was taken at each of the following points on the curve: (1) initial weight, (2) weight after 1 week's feeding, (3) weight after 2 weeks' feeding, (4) weight after 3 weeks' feeding, (5) minimum weight after cessation of feeding, (6) maximum weight after absorption of water, (7) weight 1 week after moulting, (8) weight 3 weeks after feeding recommenced.

The fresh and the dry weights were determined. The fresh weights followed the typical moulting curve (Fig. 4). The dry weight increased during the initial 2 weeks and then fell fairly rapidly until the eighth week. During the period of absorption of water very little dry matter was lost, but during elimination the amount lost was greatly increased. About one-quarter of the loss of dry matter during this final period was due to the exuviae.

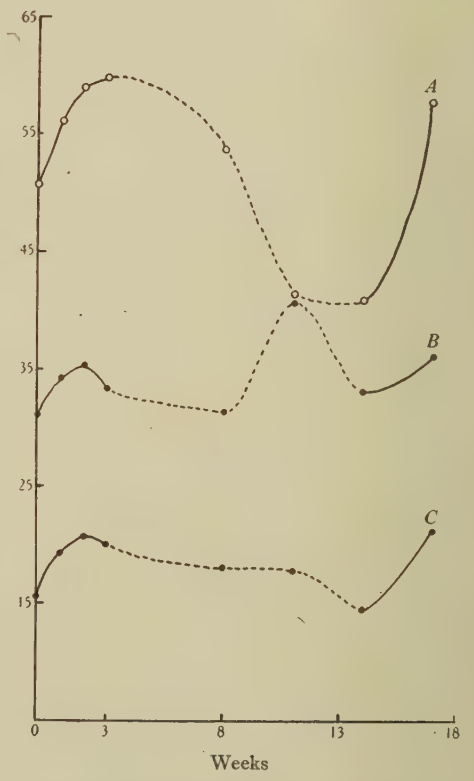

Fig. 4. Changes in the dry-matter content and percentage dry weight of medium-sized wireworms during the moulting period. A, percentage dry weight. B, fresh weight in mg. C, dry weight in mg.

As a result of these changes in the dry-matter content of the wireworm, its percentage dry weight does not fluctuate to the extent originally expected; it simply increases to a maximum at cessation of feeding, falls to a minimum at the point of maximum absorption of water, when it remains constant until feeding starts again: it then increases rapidly. The amount of dry matter lost during this moult—nearly 30%—is very great compared with the initial amount present.

During the short period of feeding observed after moulting in this experiment, the mean fresh weight of the wireworms increased by 3·3 mg., but the amount of dry matter increased by 6·4 mg. The wireworms must therefore have lost approximately 3 mg. of the water which they contained 1 week after moulting, this being replaced by dry matter. This result explains the observation, made many times, that a wireworm can remain constant in weight or even decrease in weight, although it has clearly consumed a very large amount of food relative to its size. The dry matter retained has replaced an equivalent amount of water.

Feeding and growth

Balachowsky & Mesnil (1935), Langenbuch (1932) and Subklew (1935) maintained that wireworms of all sizes can feed on decomposing organic matter or humus in addition to living vegetable material. Miles & Cohen (1941) reported that just-hatched wireworms kept in soil without added plant food appeared to feed on organic matter such as pieces of decayed roots. Their growth was slower than that of those receiving wheat grains or couch grass stems as food, and they generally died while still in the second instar.

A preliminary experiment suggested that half-grown wireworms, 20–25 mg. in weight, cannot utilize decaying organic matter in the soil. A critical experiment was set up to investigate the matter further. Soil was taken from one of the Barnfield mangold plots at Rothamsted which have not received any organic manures for 60 years and was, therefore, very poor in organic matter. Organic matter in various stages of decay was added to this soil to bring the organic matter content up to 5%: sewage sludge, farmyard manure and freshly cut grass was used. Three controls were introduced into the experiment, a rich potting soil, Barnfield soil without added organic matter, and Barnfield soil with wheat. Individual wireworms were confined in pint water-culture bottles with 100 g. of the various soils which had their water content adjusted to and maintained at 45% saturation: twenty replications of each treatment were set up. The experiment was terminated after 10 weeks. The wireworms were weighed fresh and their dry weights were determined (Table 1).

Table 1. *The utilization of organic matter by half-grown wireworms*

	Original	Barnfield soil	Potting soil	Barnfield soil + sewage sludge	Barnfield soil + farmyard manure	Barnfield soil + rotting grass	Barnfield soil + wheat
% increase in fresh wt.	—	5·9	7·7	1·2	6·1	6·9	14·1
% dry wt.	42·4	31·0	29·0	29·5	32·7	28·7	44·4
Change in amount of dry matter, mg.	—	−2·2	−2·8	−3·1	−1·9	−2·3	+2·1

The mean percentage increases in fresh weights of all the treatments except sewage sludge show a significant increase over the original weights, while that for wheat is significantly greater than all the others (S.E. = ±0·74). From these results it might be concluded that organic matter in the soil could be utilized to a small extent for growth. However,

examination of the dry weight results shows conclusively that wireworms confined in soil rich in organic matter lost dry matter to the same extent as those confined in soil very poor in organic matter. The wireworms having wheat as a source of food increased their dry-matter content considerably.

A similar experiment was carried out with newly hatched wireworms. These were confined in Barnfield soil alone and Barnfield soil to which was added either wheat grains, carrot disks, sewage sludge, farmyard manure, or small pieces of lawn turf. The wireworms were examined after 3 months. The mean weight of those fed on wheat was ·44 mg., on carrot ·37 mg., on decaying turf ·25 mg., S.E. ± 0·023. The difference in the means of those fed on wheat and carrot is barely significant, but the mean of those fed on decaying turf is significantly lower than the others. None survived on either the soil alone or soil with sewage sludge or farmyard manure, 6% survived on decaying turf, 62% on carrot and 73% on wheat.

Stone's (1941) observations on the growth of wireworms of *Limonius californicus* Mann. reared on wheat, corn, and lima bean seeds indicated that there was little, if any, difference in the rate of growth on the three kinds of food, but that the adults resulting from the wireworms fed on lima beans were much smaller than those resulting from the wireworms fed on corn or wheat.

In order to test the relative suitability of some of the foods used in the present experiments, large wireworms were allowed to feed on carrot disks, wheat, and bean seeds for 34 weeks, with starving individuals as controls, and the results given in Table 2 were

TABLE 2. *The effect of various foods on the increase in fresh weight of wireworms*

	Starving	Bean	Carrot	Wheat
Mean change in fresh wt. in mg.	−3·8	2·5	8·7	16·1

S.E. ± 1·13.

obtained. Wheat is clearly the most suitable food for rapid growth, with carrot and bean less suitable, but the former significantly better than the latter. The results of an unfinished experiment indicate that the increase in dry matter is greater on carrot and wheat than on bean. Accurate methods for determining the amounts of the various foods consumed by a wireworm have not yet been worked out, so that it is not possible to say with certainty whether the rapid growth on wheat is due to the quality or to the quantity eaten, or both. It has, however, been established that the wireworm feeds for a longer period on wheat than on carrot or bean seed. During the 34 weeks' observations under review the wireworms fed for an average of 9·4 weeks on carrot and bean seed but for 14·3 weeks on wheat; the S.E. is ± 0·6. This difference in length of the feeding periods of large wireworms on wheat and carrot does not hold for small wireworms weighing initially 5–7 mg. During an experiment which has continued for 27 weeks they have fed for half that time on both wheat and carrot but have increased in weight significantly more on wheat than on carrot.

DISCUSSION

The results obtained indicate that a suitable critical technique has been evolved for a study of the wireworm in relation to its physical environment, and they also may make it possible to suggest explanations of certain field observations such as the occurrence of the spring

and autumn periods of damage, and the fact that wireworms in one field may have stopped feeding but may continue to feed in an adjacent one. In the first instance the explanation may lie, at least in part, in the relationship of the fasting periods at moulting time to the seasonal trend of temperature, in the second instance to the suitability of their food in the previous year. In soil insecticide tests carried out by Gough (unpublished) considerable variation in mortality was found between the replicates. It is possible that this variation was due to the varying physiological state of the wireworms used: also that the degree of the permeability of the cuticle in relation to the moulting cycle may be an important factor. These and other problems are being studied.

Summary

A technique has been worked out for rearing wireworms which permits detailed studies of individuals in relation to food, soil moisture, and other factors. As the wireworm increases in size the moulting process becomes more elaborate. A large portion of the life of the wireworm is spent in non-feeding phases. Newly hatched and half-grown wireworms do not utilize decaying organic matter in the soil. The type of vegetable food on which the wireworm feeds influences its rate of growth.

REFERENCES

Balachowsky, A. & Mesnil, L. (1935). *Les insectes nuisibles aux plantes cultivées*, **1**, 760. Paris.

Bolas, B. D. & Melville, R. (1933). The influence of the environment on the growth and metabolism of the tomato plant. I. Methods, technique and preliminary results. *Ann. Bot., Lond.*, **47**, 673–88.

Keen, B. & Raczkowski, H. (1921). The relation between the clay content and certain physical properties of a soil. *J. agric. Sci.* **11**, 441–9.

Langenbuch, R. (1932). Beiträge zur kenntnis der biologie von *Agriotes lineatus* L. und *Agriotes obscurus* L. *Z. angew. Ent.* **19**, 278–300.

Miles, H. W. & Cohen, M. (1941). *Report for* 1939 *and* 1940, *Entomological Field Station, Warburton, Cheshire.*

Stone, M. W. (1941). Life history of the sugar-beet wireworm in Southern California. *Tech. Bull. U.S. Dep. Agric.* no. 744.

Subklew, W. (1935). *Agriotes lineatus* L. und *Agriotes obscurus* L. Ein beitrag zu ihrer morphologie und biologie. *Z. angew. Ent.* **21**, 96–122.

Wigglesworth, V. B. (1939). *Principles of insect physiology*. London: Methuen.

Woodworth, C. E. (1938). The reactions of wireworms to arsenicals. *J. agric. Res.* **57**, 229–38.

(*Received* 10 *October* 1941)

WIREWORMS AND AGRICULTURE, WITH SPECIAL REFERENCE TO *AGRIOTES OBSCURUS* L.

By HERBERT W. MILES, D.Sc., Ph.D.
University of Manchester

Wireworms are the elongate, yellow, smooth-skinned larvae of click beetles, and the species most widely distributed and generally destructive in Britain is *Agriotes obscurus* L. (Ford, 1917; Cohen, 1942). This species has a long life cycle and spends 4 years as a wireworm in the soil. Wireworms are usually found in greatest numbers in the soil of grassland where the static conditions favour their survival. In the cycle of varying conditions in land under arable culture, unfavourable temperature and moisture conditions occur periodically, the food supply is dispersed and reduced, and the insects may be disturbed at critical periods of their development and exposed to weather and natural enemies.

Of the land in agricultural occupation, grassland contains the greatest variety of plant species and the densest plant populations, and the nature of the herbage is such that the feeding of wireworms makes little immediate change in the botanical composition or in the numbers of plants. During the 4 years of larval life the wireworms feed on the underground parts of grassland plants, burrowing into stems and biting into the bases of grasses just above their growing points. They destroy tillers here and there, and move about in the soil travelling towards the surface or away from it in response to climatic changes and the need for food and protection. Conditions in grassland are so stable that the majority of the wireworms remain in the top 6 in. of soil throughout the greater part of the year.

When mature and about 1 in. long the wireworms make their way into compact soil, usually 3–4 in. below the surface, and by rotating they fashion cells in which the prepupal, pupal and early adult stages are passed. During the prepupal period the wireworm contracts and becomes swollen and pale along its sides, and in 2–3 weeks the integument is shed and the soft white chrysalis exposed. In the course of the next few weeks the chrysalis hardens and darkens, and by the end of September most of the pupal cells contain young adults which have shed the transparent enveloping membranes of the chrysalis stage. These young adults usually remain in the soil cells until spring when, from March onwards, they make their way to the surface.

From March to June click beetles may be found at the surface of the soil. They move about mainly at night during moist weather and feed upon the leaves of grasses and grassland plants. During the day they hide under vegetation and in soil crevices.

Eggs are laid in batches in the surface soil during April, May and early June, either in damp places or during showery weather. They are nearly spherical and about $\frac{1}{50}$ in. diam. A beetle lays 40–100 or more eggs. The eggs take 5–6 weeks to incubate, and young wireworms hatch in June and July.

The young wireworms soon begin to feed, and they may burrow into the tissue of the underground stems of grasses, notably couch grass, *Agropyrum repens*. Where batches of eggs have occurred numbers of young wireworms may be found close together in the soil, and at Warburton thirty-two young larvae have been taken within the confines of a 4 in.

diam. soil sample. As the wireworms grow they disperse and become distributed throughout the soil.

During succeeding years new generations of wireworms are added to the soil so that the wireworm population of an area is made up of insects of the first, second, third and fourth year groups. The size of the different age groups reflects in some degree the numbers of adults present and the suitability of conditions for egg laying, incubation and the survival of young larvae during particular years. Fluctuations in the wireworm population of grassland pass unnoticed as there is no readily visible association between these fluctuations and the condition of the grassland herbage.

When grassland is ploughed for arable cultivation the whole environment is fundamentally changed. The effect on the wireworms is cataclysmal: their food supply is decreased, the land is periodically disturbed, and young beetles in soil cells are roughly ejected. The beetles are able to crawl or fly to a more favourable environment, but the wireworms have to endure the change. By adapting themselves to the altered conditions and feeding on the crops the wireworms have provided agriculture with one of its most difficult problems (Miles & Cohen, 1939).

Field work on wireworms in the north-west of England

In the north-west of England wireworm injury was particularly severe on potatoes during the years 1934–6, and the need for local research on wireworms was generally recognized. Through the co-operation of farmers and agricultural interests and with the support of the Ministry of Agriculture and Fisheries, it was possible in 1937 to take a field at Warburton, Cheshire, for a study of wireworms (Miles & Cohen, 1938). The field had been neglected for several years and had tumbled down to grass. It was a wilderness of couch grass, Yorkshire fog and bent grass, with bracken encroaching from the hedge sides. The soil was sandy and very acid (*p*H 5·18), and the total nitrogen (0·207%) and loss on ignition (6·93%) indicated a moderately low moisture and base-holding capacity, and it was also deficient in available potash (Stewart, 1938). When sampled in April 1937 by hand-sifting, the number of wireworms was found to be about 300,000 per acre, and *Agriotes obscurus* L. comprised 96% of the population (Cohen, 1942). This estimated population does not represent the total number of wireworms in the soil, as the hand-sifting method of examination of the soil usually only discovers wireworms in the second, third and fourth years of larval life. Since these are the wireworms associated with injury to farm crops the estimated population may be taken as the effective wireworm population.*

The experimental field at Warburton was tractor-ploughed and laid out in a series of plots each about $\frac{1}{20}$ acre. These plots were cropped with farm and market-garden crops usual in the district, and the basis of the cropping system was the local four-course rotation: cereals → rye-grass and clover → cereals → potatoes. The crops received the usual routine cultivation and manuring. The soil of the plots was sampled annually during the autumn and winter to obtain estimates of the wireworm populations. By comparing the estimated wireworm populations from year to year, the effects of cropping and tillage could be discerned. From 1939 the small plots were grouped and cropping unified over

* It is the effective wireworm population that is the immediate concern of the farmer and the agricultural entomologist, and in survey work undertaken by advisers in agricultural entomology the term 'wireworm population' usually refers to the effective wireworm population.

larger areas and plots under ley were left for longer periods. The cropping of typical pairs of plots and the changes in the wireworm population from year to year are shown in Table 1.

TABLE 1. *Cropping and associated wireworm populations: Warburton, Cheshire, 1937–41*

	Year	Crop	Wireworms per acre	
Plots F and H	1937	Grass	—	—
	1938	Grass	370,000 ± 43,000	235,000 ± 30,000
	1939	Potatoes	67,000 ± 14,000	147,000 ± 20,000
	1940	Wheat	169,000 ± 24,000	108,000 ± 22,000
	1941	Grass	145,000 ± 19,000	169,000 ± 20,000
Plots B and E	1937	Roots	199,000 ± 21,000	317,000 ± 21,000
	1938	Potatoes	98,000 ± 14,000	145,000 ± 21,000
	1939	Oats	20,000 ± 8,000	63,000 ± 11,000
	1940	Grass	137,000 ± 38,000	127,000 ± 22,000
	1941	Grass	284,000 ± 34,000	222,000 ± 40,000
Plots C and D	1937	Roots	193,000 ± 21,000	183,000 ± 30,000
	1938	Cereals	—	—
	1939	Grass	76,000 ± 12,000	88,000 ± 14,000
	1940	Grass	35,000 ± 8,000	131,000 ± 19,000
	1941	Grass	269,000 ± 31,000	194,000 ± 27,000
Plots O_1 and O_2	1937	Green manure	186,000 ± 30,000	108,000 ± 20,000
	1938	Potatoes	76,000 ± 13,000	94,000 ± 20,000
	1939	Wheat	41,000 ± 9,000	45,000 ± 13,000
	1940	Grass	76,000 ± 10,000	76,000 ± 13,000
	1941	Potatoes (O_1)	20,000 ± 6,000	
		Oats (O_2)		116,000 ± 21,000

On plots B and E there was a decrease in the wireworm population during the 2 years the land was under roots, and the decrease was continued in 1939 when after the cereal crop the wireworm population reached its lowest point. During the 2 years under ley the wireworm population again increased. In plots O_1 and O_2 the crop sequence of green manure and potatoes and wheat reduced the wireworm population. On plot O_2 ley, 1940, followed by oats, 1941, was associated with an increase in the numbers of wireworms, while on O_1 though there was an increase in wireworms associated with ley in 1940 a decrease was recorded after potatoes in 1941. Plots F and H showed a decrease in the numbers of wireworms following the cultivation of potatoes as a first crop after ley. Plots C and D showed the building up of the wireworm population during 3 years under ley.

Influence of cropping and tillage

From these observations it appears that fluctuations in the wireworm population of a field are closely linked with the sequence of cropping. Under arable cultivation with its cycle of such operations as disking, ploughing, rolling and harrowing, the numbers of wireworms in the soil tend to decrease year by year. The decrease is most marked during the cultivation of root crops. These have the lowest plant populations found on agricultural land other than fallow, and they leave much of the surface of the soil bare until well into July. Land under root crops affords little shelter to the click beetles and is not usually selected for egg laying. The soil conditions under root crops appear to be so unfavourable to wireworms that the decrease in the wireworm population is frequently much greater than can be accounted for by the maturation of the fourth-year wireworms.

Cereal crops have a moderately high plant population, particularly when seeded down, and afford some concealment and protection to click beetles, to their eggs during incubation and to young larvae. Where cereals are followed by ley or by a second cereal crop seeded down the wireworm population may increase. When a period under arable culture is succeeded by a period under ley, the wireworm population decreases while the land is under the plough and increases again during the ley period. Where the ley remains for less than 3 years the wireworm populations are generally low, but when leys remain for 5 or more years high wireworm populations may be built up.

The basis of the cropping of arable land over most of England is the four-course rotation: cereals → roots → cereals → clover. This rotation is often modified to suit local conditions. The clover is frequently left as ley for a short period and then two cereal crops are taken together, or a crop of potatoes or brassicae is taken after the root crop. In some districts the 4-year cycle is reduced by the omission of a cereal crop and this three-course rotation is sometimes extended by the insertion of potatoes or green crops after the root crop and then the ley is left down for a period. Under the usual four-course rotation in mixed farming the most favourable conditions for infestation by wireworms occur during the cereal-clover period, but since it is of short duration high wireworm populations do not develop and wireworm injury is generally slight.

Influence of local conditions

Rotations, local practices or other local conditions may be responsible for local fluctuations in wireworm intensity or in the amount of injury associated with different levels of wireworm population. In north Cheshire, where sandy soils and peaty sands predominate, a four-course rotation consisting of cereals → rye-grass and clover → cereals → potatoes is followed. There is a local shortage of farmyard manure, and what little stable manure is obtainable is made with sawdust 'bedding'. Only light dressings of these organic manures are available for the potato crop. A second cut of hay is taken so that usually there is little bulk of vegetable matter when the clover root is ploughed in, and this is largely exhausted by the cereal crop which follows. The frequent exposure of the surface soil during the cultivation incidental to the potato crop keeps the surface few inches of soil very dry. When rain falls it drains away rapidly, often without wetting the bulk of the soil, and comparatively little moisture is retained except in the immediate neighbourhood of the root mass. Under these conditions of humus deficiency and lack of soil moisture near the surface extensive wireworm injury to potato tubers occurs even when the wireworm population is low. In eight fields where the wireworm population was 100,000 or less to the acre the percentages of tubers damaged by the insects were respectively 25, 20, 26, 42, 35, 48, 33 and 54.

Summary

The life cycle of *Agriotes obscurus* L., the larva of which is the common wireworm of agriculture in Britain, is reviewed. The influence of rotation and cultivation on the wireworm population of agricultural land is instanced by the histories of eight plots with diverse cropping at the Warburton Field Station, Cheshire. The wireworm population tended to decrease while the land was under fallow crops and to increase when the land

was under ley. Local conditions may influence the amount of injury caused by particular wireworm populations. In north Cheshire serious injury to the potato crop is often associated with low wireworm populations.

REFERENCES

COHEN (1942). Observations on the biology of *Agriotes obscurus* L. I. The adult insect. *Ann. appl. Biol.* **29**, 181.

FORD (1917). Observations on the larval and pupal stage of *Agriotes obscurus* L. *Ann. appl. Biol.* **3**, 97.

MILES & COHEN (1938). Investigations on wireworms and their control: 1st Rep. *J.R. Lancs. agric. Soc.* p. 58.

MILES & COHEN (1939). Investigations on wireworms and their control: 2nd Rep. *J.R. Lancs. agric. Soc.* p. 53.

STEWART (1938). Observations on the soil of the Warburton Experimental Field. *J.R. Lancs. agric. Soc.* p. 69.

(*Received* 10 *October* 1941)

OBSERVATIONS ON THE BIOLOGY OF *AGRIOTES OBSCURUS* L.

I. THE ADULT INSECT

By MORRIS COHEN, M.Sc., Ph.D.

Department of Agricultural Entomology, University of Manchester

(With 8 Text-figures)

As part of a general investigation on wireworms and agriculture (Miles & Cohen, 1938) observations have been made on the adult of *Agriotes obscurus* L., the common click beetle in the north-west of England. The various records from which the present account has been prepared have been made chiefly at the Warburton Field Station of the Agricultural Advisory Department of the University of Manchester. Supplementary notes have been made elsewhere in Lancashire and Cheshire or abstracted from the records collected by members of the entomological Advisory Staff in the course of routine soil-sampling work.

To obtain information on click beetles the insects were trapped on grassland by removing 12 in. squares of turf and filling the cavities so made with dried couch-grass roots. These roots were shaken out daily and the beetles collected. Beetles were also obtained by examining tufts of turf on ploughed fields, and on the undersides of sacks and potato sprouting trays left on the land after planting.

To observe mating and oviposition beetles were kept in batches of eight in glass tubes (3 × 1 in.) half-filled with sifted damp soil. Pieces of the leaves of cereal plants were introduced for food. The sexes were determined by post-mortem dissection. The eggs were incubated in these tubes, and whenever possible the newly hatched larvae were counted to determine the exact number of eggs laid. The larvae were reared in the tubes which were periodically examined, when grains of wheat were introduced for food and moulted skins removed for measurement of head-capsule dimensions. More recently, in 1941, climatic data have been collected through the installation of a Meteorological Station, and continuous records of soil temperature have been obtained by means of a two-bulb recording thermometer.

1. Distribution of *Agriotes obscurus* in Lancashire and Cheshire

A. obscurus occurs commonly throughout the whole of England and Wales. The main factors influencing numerical proportion of the species in wireworm populations are altitude and the type of flora. Increase in altitude involves in itself changes in soil type and in the constitution of the plant environment. Estimation of the prevalence of the species was more easily and accurately achieved by sampling for the larval stage, and most of the data given in Table 1 were derived from counts of larvae obtained in soil samples. At Warburton, however, the adults were trapped in the period 1937–40, and while the figures given are indicative of the make-up of the population they do not agree closely with the data obtained by soil sampling for the larvae. The variations can be ascribed to differences in the habits of the adult of the various species encountered.

TABLE 1. *Occurrence of* Agriotes *spp. at Warburton*, 1939–40

	Larvae		Adults	
Year	Total examined	% *Agriotes*	Total trapped	% *Agriotes*
1937	621	95·8	64	68·8
1938	2768	98·8	445	87·6
1939	1633	94·8	505	75·0
1940	961	60·8	169	71·6

TABLE 2. *Occurrence of* Agriotes *spp. in the wireworm population in Cheshire*

Region	Average altitude (ft.)	*p*H	P_2O_5	K_2O	% *Agriotes* spp.
North	200	5·74	0·018	0·008	73·8
Central	150	5·65	0·018	0·008	100·0
East	300	5·23	0·015	0·011	73·3
South	150	5·84	0·019	0·008	100·0
South-west	50	6·16	0·024	0·014	100·0
Wirral	150	5·50	0·009	0·010	100·0

TABLE 3. *Occurrence of* Agriotes *spp. in the wireworm population in Lancashire*

Region	Average altitude (ft.)	*p*H	P_2O_5	K_2O	% *Agriotes* spp.
North	200	5·26	0·018	0·017	84·9
Fylde and Central	120	5·26	0·019	0·018	99·4
East	500	5·04	0·016	0·013	20·0
South	180	4·77	0·018	0·015	58·1
South-west	60	4·90	0·018	0·015	83·7

In Cheshire, records obtained during 1940–1 by the entomological staff of the Agricultural Advisory Department indicated that 95% of the wireworms found belonged to the species *A. obscurus*, while in Lancashire this species comprised 76% of the wireworms collected. Tables 2 and 3 give the composition of wireworm populations in Cheshire and Lancashire respectively, together with the average altitude, *p*H, and available soil phosphates and potash for the respective districts in each county. Fig. 1 shows the areas into which the two counties were divided for the purpose of compiling the tables. It will be seen that except in north and east Cheshire the common wireworm is dominant. In these two areas most of the other species of wireworms found in grassland occur.

2. HABITS OF THE ADULT

(1) *Appearance on the surface.* After emergence from the chrysalis the adults generally remain in the soil throughout the winter and appear on the surface in the spring. There is some evidence that beetles may leave the soil in the months after transforming from the chrysalis and pass the winter 'in tufts of grass, hedge bottoms or refuse of dykes' (Board of Agriculture leaflet No. 10, 1918). Rymer Roberts (1919) stated that *A. obscurus* left the pupal cell and 'hibernated in the neighbouring soil', but noted that other observers had found the mature beetle in the pupal cell in winter and early spring. During the present investigation many records were accumulated which indicated that adults of *A. obscurus* generally remained in the soil throughout the winter and spring. In numerous cases the

beetle was found together with the last larval skin showing that hibernation occurred either in or close to the pupal cell.

The date on which the beetles end hibernation either in the soil or in surface vegetation and become numerous on the surface is dependent on climate and weather conditions. Observations at Warburton indicated that the last week of March initiated the period of emergence of the beetles. In 1937 observations commenced in May and on 21 May the beetles were found in numbers under stones and tufts of turf in fields of oats and wheat. In 1938 the first record was obtained on 31 Mar., when twenty-nine beetles were collected. In 1939, 1940 and 1941, the beetles appeared on the surface in the first week of April; they were obtained in numbers on 11 and 12 Apr. 1939. These dates are considerably earlier than those quoted by Rymer Roberts (1919) who in observations in Hertfordshire over a 4-year period recorded few beetles before 15 May.

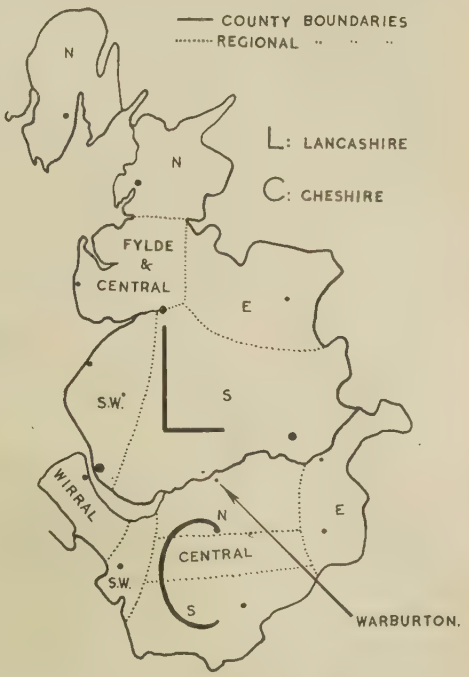

Fig. 1.—Map of Lancashire and Cheshire showing divisions into regions for the wireworm survey.

In Switzerland, Guéniat (1934) recorded the capture of *A. obscurus* on 16 and 22 Mar. 1933, although in the years 1931 and 1932 his earliest captures were made on 4 and 9 Apr. respectively. In the same country Neuweiler (1926) recorded only a single specimen in the first half of March during 1915–25. In Poland, Chrzanowski (1927) found the first emergences of this species in 1923 on 23 Mar. and in France, Mesnil (1930) and Regnier (1928) recorded the first captures of *A. obscurus* in early April. Though the climatic conditions of these countries and England differ considerably, there is a remarkable agreement between the dates on which the adults first appear on the surface.

(2) *Activity of the adults on the surface.* The daily meteorological conditions in any particular area are the controlling factors in the activity of the beetles. Thus Adrianov (1914) stated that at the end of May adults of *A. obscurus* and *A. sputator* were very active in bright sunlight on the stems and ears of wheat and rye. At sunset, and when the weather was cold and windy, the beetles hid in the grass under lumps of soil. Rymer Roberts (1919) stated that he had obtained very few beetles of these species by sweeping cereals but considerable numbers were found under heaps of cut grass, particularly in dull and showery weather. The best data available for the continent are given by Guéniat (1934) and are valuable for comparison with data accumulated at Warburton in 1938 and 1939. He stated that the daily frequency of the beetles was directly related to temperature and humidity. Full meteorological data were not given, but his results show that after warm spring weather and light rain many more beetles were collected than after cold, windy weather with snow. Data obtained in 1931 and 1932 on the same field showed that in the latter

year when the weather was very adverse, with snow, cold wind and rain, only 102 *A. obscurus* were collected in the season, the previous year's total being 1458, the weather being much warmer. At Warburton full records were obtained in 1938. The trapping method mentioned earlier was employed and the same number of traps were examined daily. These traps were not necessarily attractive to the beetles but served to obtain a measure of their normal activity; it was much easier to examine the loose material in the traps than to search the roots of grasses. Fig. 2 shows the numbers of beetles taken in the traps in 1938 in 4-day periods together with the average morning (9 a.m. G.M.T.) and evening (9 p.m. G.M.T.) relative humidities and average grass minimum temperature for the same periods. The

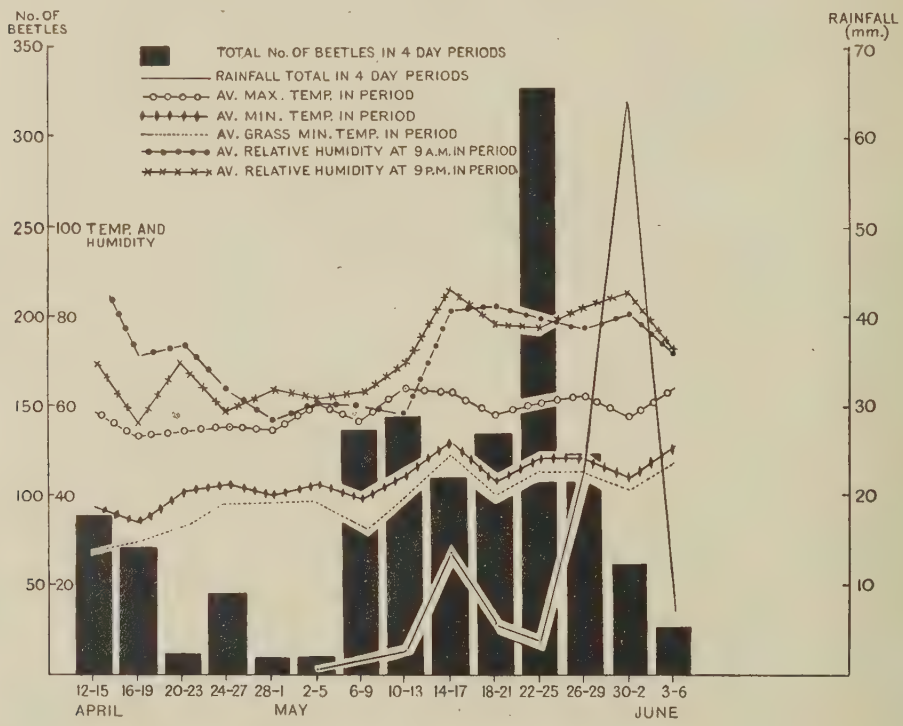

Fig. 2. Activity of *A. obscurus* L. in relation to daily weather conditions in 1938.

rainfall is given as a total for the 4-day period. Most beetles were taken during the periods in which rain fell and when the relative humidity of the atmosphere was high; the rain periods appeared to follow the peak periods for the insects. The temperature of the atmosphere also showed an increase through the period of the trapping and the highest temperatures were recorded during the peak periods. In 1939 fewer beetles were taken in the traps and there was a long period when the traps were not examined. The available data again indicated that most beetles were taken during the periods in which there was rain and that the relative humidity was highest during the periods when the beetles were most active. The temperature data during 1939 were not as informative as in 1938, and the trapping data in 1938 showed that the beetles were most active and numerous in a well-

defined period. While further observation is required, it would appear that humidity, rain and to a lesser degree temperature, influence the activity of the beetles.

The relative humidity of the atmosphere about the bases of plants must vary considerably and may be very different from the relative humidity of the atmosphere several feet above the surface of the soil. Thus Stocker (1923) found that the relative humidity at 2 cm. above the soil in a pasture near Freiburg in Germany, was 96%, while at 13 cm. above the soil and still in the vegetation the relative humidity was 78% compared with 57% at a height of 100 cm. above the surface of the soil. These records were obtained in July on a quiet sunny day. A few records obtained in May 1941 of the relative humidity in the vegetation of a grass plot at Warburton are given in Table 4, with the values for the open air for comparison. The readings in the vegetation were made at a height of 5 cm. above the surface of the soil.

TABLE 4. *Comparison of relative humidity* (A) *in the herbage layer of grassland and* (B) *in the atmosphere 4 ft. above soil level. May* 1941

	9 a.m.		1 p.m.		5 p.m.	
Date	A	B	A	B	A	B
13 May	100	78	90	64	88	63
14 ,,	100	82	84	62	94	66
19 ,,	100	67	82	70	100	62
20 ,,	100	54	77	43	100	59
21 ,,	100	54	75	68	100	82
22 ,,	100	64	90	68	100	80

The relative humidity of the air just above the surface of fallow land is always less than that just above soil which is covered by vegetation, due to the transpiration of the plants. Where soil supports a reduced plant population as in the case of row crops and the early stages of cereal crops, the relative humidity of the atmosphere in the vegetation while greater than that above fallow land is still less than that in the vegetation zone of grassland.

It is reasonable to infer that the preferred habitat of the beetles is one in which the relative humidity is high. This condition obtains in habitats with high plant populations, and the commonest such environment is grassland.

3. Period of day during which movement occurs

Movement on the surface and on vegetation. While *A. obscurus* has been observed moving on the surface of ploughed land during the daytime such records have been very few, and generally the beetle avoids light and moves chiefly during the evening and night. Trapping indicated that the insect was most commonly found during the early morning, and during the remainder of the day the traps yielded few specimens. Similarly, the early morning inspection of the undersides of stones, turf, potato sprouting boxes, and pieces of sacking laid on the grass, was far more productive of beetles than examinations of the same sites during the rest of the day. Movement therefore takes place chiefly during the night, and this nocturnal movement is primarily due to negative phototropism, humidity and temperature. Hygrograph records during April and May indicated that the relative humidity of the atmosphere was high during the night period (12 midnight to 8 a.m.), while from 8 a.m. to 2 p.m. the relative humidity dropped sharply. Fig. 3 shows a record of the relative humidity of the atmosphere obtained during the week beginning 5 May 1941.

A similar daily variation of less range occurs in the atmosphere in vegetation and Table 4 gives some records obtained in 1941. While the temperature of the atmosphere over and in vegetation also varies during the day, the lowest temperatures always tend to be higher than those recorded when the beetles first appear on the surface, and it is thought that the temperature factor is secondary to humidity. Under artificial conditions in a glasshouse, beetles were observed crawling about in the period 12 noon–3 p.m. The atmosphere was extremely moist, and the air temperature was between 65 and 70° F.

On the Continent accounts of the movement of the insect vary. Thus Chrzanowski (1927) stated that beetles could be found on plants about midday, and that in the hottest period of the day they remained on the ground where they crawled about between the plants. He also suggested that in the period 3–6 p.m., the beetles began to travel over the fields in search of favourable sites for oviposition. Adrianov (1914) noted that movement occurred freely during bright sunlight. No meteorological data were given by either of these workers. During the period 1936–41 *A. obscurus* was never taken on the aerial stems and leaves of plants and systematic use of the sweep net failed to recover a single specimen. It is evident

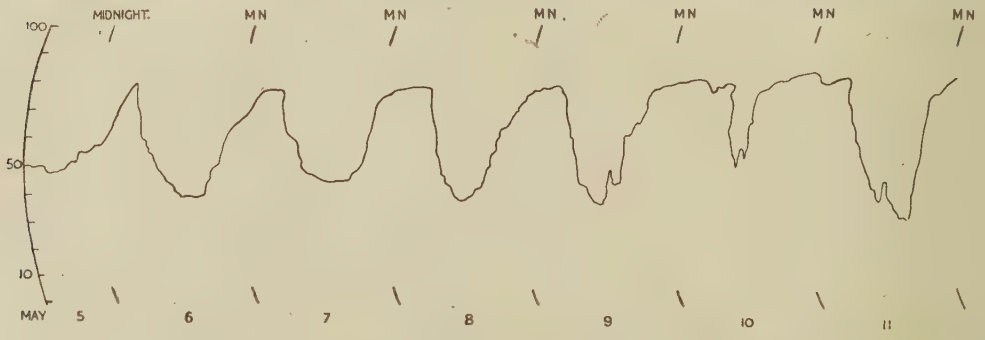

Fig. 3. Relative humidity of the atmosphere during week beginning 5 May 1941. MN = midnight.

that the beetle does not readily climb on vegetation, and further support for this conclusion is provided by Langenbuch (1932) who stated that he had never collected the insect on the foliage of any plants and by Guéniat (1934) who found it only twice on foliage.

Flight. No record was obtained of this species flying during the period 8 a.m. to 6 p.m. Fryer (1941) stated that he had taken *A. obscurus* flying in the late evening in June under conditions of high humidity with little wind. Similarly, Britten stated (unpublished MS.) that he had often observed the beetle flying in the very early morning (4–5 a.m.) in the same month under similar weather conditions, close to the river in the Eden Valley in Cumberland. Both these records are from environments close to water and probably with conditions of high humidity. Pospelova (1939) working in Russia stated that *A. obscurus* was on the wing from the end of May until the beginning of July, the maximum flight period being in the latter half of June. No mention was made of the weather conditions.

In an attempt to trap click beetles by means of a chemical attractant, traps containing a mixture of geraniol and eugenol were used in 1938. These two essential oils have been used with great success to trap the Japanese beetle, *Popillia japonica* Newm., in the U.S.A. A trap similar to that used in the U.S.A. containing a mixture of geraniol and eugenol

was suspended at a height of 30 in. over a grass plot where beetles were occurring in numbers. The insects caught were mainly Diptera with a small number of humble bees and Staphylinid beetles. The total numbers were not very great, and not a single specimen of *A. obscurus* or indeed any other Elaterid was present in the catch. It is possible that the weather conditions were not suitable for the chemicals to exert the optimum degree of attractiveness, temperature being one of the controlling factors. In studies of baits for trapping the codling moth in the U.S.A., Steiner (1929) reported that numbers of Elaterids were attracted to ethyl proprionate in a honey and sugar solution, and Hawkins (1936) in Maine, U.S.A., recorded the flight of *A. mancus* Say at a height of 15–36 in. above soil-level between 10 a.m. and 2 p.m.

In view of the variation in the density of wireworm populations and since wireworms themselves do not move long distances, flight of the adults would appear to be the main method of dispersal of the insect and of the initiation of new centres of infestation. Lack of observations, especially those with relevant meteorological data, makes it impossible, however, to state whether flight in *A. obscurus* is of common occurrence.

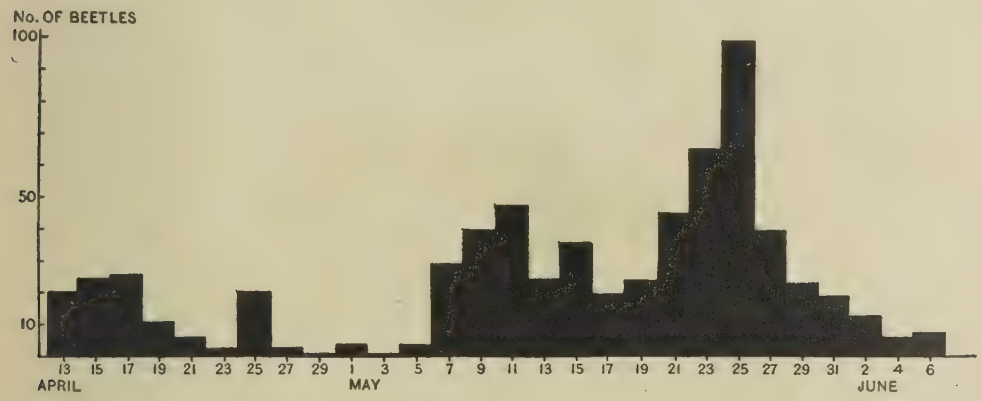

Fig. 4. No. of adults of *A. obscurus* L. taken in traps in 1938.

4. Peak occurrence of the adult

The trapping records for 1938 show that the majority of beetles were obtained in the period 15–28 May (Fig. 4), with considerable numbers also at the beginning of April and in the first week of May. In 1939 most beetles were collected from 1 to 27 June, and in 1940 the maximum numbers occurred during the second and third weeks of May. It would appear that in the north-west of England the peak period of occurrence is in the latter half of May and the first half of June.

5. Food of the adult

Feeding was not observed in the field because of the habits of the beetles. Mesnil (1930) stated that the adults of *Agriotes* spp. were carnivorous, and that if given a choice of animal and vegetable foods the animal food was always preferred. He noted that in the absence of animal food the three species of *Agriotes* fed on plant matter. Guéniat (1934) only once

recorded beetles eating in the field, and the food consisted of the seeds of spring oats about to germinate.

In the progress of this work feeding under laboratory conditions was often observed. The insects fed voraciously after their appearance on the surface, although adults which overwintered in the laboratory did not feed throughout the winter and spring. The food offered consisted of portions of the leaves of oats, wheat, rye grass, cocksfoot and red and white clover. The graminaceous plants were preferred and the clovers were rarely eaten. The beetles macerated the leaves of wheat and oats and the grasses, reducing them to a soft green skeleton of veins.

6. Maturation of the sexes

Dissections to determine the condition of the ovaries showed that in October when the beetles were in the soil, the ovarian tubules measured 0·75 mm. in length. In the spring after emergence had occurred and feeding had been in progress for about a fortnight the tubules had increased to 1·25 mm., and shortly after oviposition had commenced, they measured approximately 2·0 mm. (These measurements do not include the germinal filament.) Maturation is therefore very rapid in the period from the end of April to the middle of May. In October oocytes are not visible in the tubules. By the end of April three oocytes are present in each tubule, and by the second week of May these have increased greatly in size and the one nearest the pedicel of the tubule is approximately the size of the fertilized egg.

7. Mating and sex ratio

Mating occurs soon after the insects become active on the surface. During oviposition experiments, further mating often occurred after the deposition of eggs had begun. Before attempting copulation the male often extruded and retracted the genitalia as though to lubricate the organ. The positions of the beetles were those normal in the Coleoptera, both sexes facing in the same direction, the male being on the back of the female. Pairs of beetles remained *in copula* from under 60 sec. to 15 min., and during the period of connexion the female often crawled actively over the soil. Guéniat (1934) noted that copulation occurred between 1.0 and 5.0 p.m., and in four records he observed the period of mating to extend to 30 min.

The sex ratio was obtained by post-mortem dissection, and it exhibited considerable variation in the years 1938, 1939 and 1941: in 1938 ♀ : ♂ :: 1 : 2·9; in 1939 ♀ : ♂ :: 1·2 : 1; and in 1941 ♀ : ♂ :: 1 : 1·3. Over the entire period the sex ratio was ♀ : ♂ :: 1 : 1·2, these figures being based on the dissection of more than 800 beetles.

8. Length of life of the adult

The beetles emerge from the pupal skin in the autumn, the majority transforming in August and September. The latest dates on which living beetles were present in the oviposition tubes were 15 June in 1938, 5 Aug. in 1939, 8 Aug. in 1940 and 11 Aug. in 1941. Collections in the field continued until 10 June in 1938, 27 June in 1939, 8 Aug. in 1940 and 11 Aug. in 1941. After these dates no living beetles were taken in the traps. Guéniat (1934) recorded that in his 1931 oviposition experiments all the beetles were dead by

10 Aug., and that in 1932 and 1933 the last dates on which living beetles were noted, were 4 Aug. and 13 Aug. respectively. It is certain that in the north-west of England the beetles do not live longer than 1 year, and that the period of activity on the surface does not exceed 5 months.

There was a considerable difference in the post-sexual life of the sexes. Generally the males die soon after mating and the first oviposition of the females, but sometimes individual males lived as long as the females they fertilized. In 1939 over 61 % of the males used in oviposition experiments had died by 15 June, while 58 % of the females died in the period 16 June–4 July. In 1941 over 83 % of the males were dead by 20 June while 65 % of the females died in the period 21 June–9 July. Fig. 5 shows the distribution of sex mortality in relation to date in 1939.

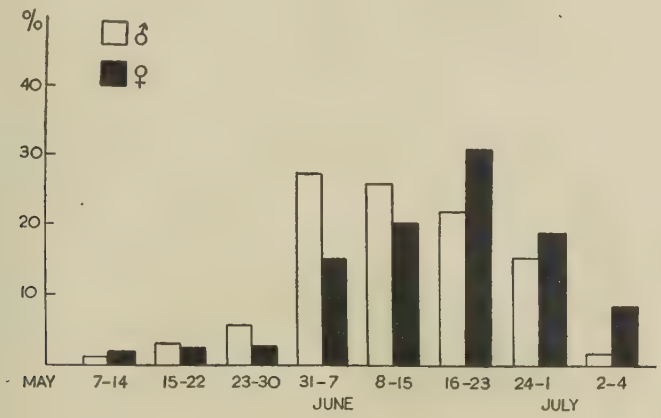

Fig. 5. Distribution of sex mortality of *A. obscurus* L. in relation to date in 1939.

9. Oviposition

During this study oviposition was not observed in the field, neither were eggs found in the soil. The habits of the beetle, particularly its nocturnal activity, make observations of this description difficult. Attempts to induce the beetles to oviposit by confining them in wide glass cylinders sunk into grassland also failed.

This difficulty in obtaining eggs under field conditions has also been noted on the Continent and in the U.S.A. and Canada. Thus Guéniat (1934) only recorded a single instance when eggs of *A. obscurus* were found in the field; they did not hatch, so that he was unable to verify his identification which was based on the size of the ova. Strickland (1933) investigating the biology of prairie-inhabiting wireworms in Canada reported that he had never been successful in causing *Ludius aeripennis* Kby. (var. *tinctus* Leconte) to oviposit in breeding cages sunk in the soil, even though many different combinations of mechanical and physical soil factors were used. In the laboratory or even in the shade of a building he experienced no difficulty in obtaining eggs.

The ease with which oviposition can be observed in the laboratory has been noted by all workers on the wireworm problem. Rymer Roberts (1919) used 4 in. pots containing grass plants, the beetles being confined by the use of glass cylinders covered with muslin.

The pots were kept in a shady place. Guéniat (1934) used seed pans containing sprouting potatoes, the pans being kept in a glasshouse. During this investigation oviposition was observed by confining the beetles in batches of eight in glass tubes. The beetles were selected according to size, the males tending to be smaller and more slender than the females. Subsequent post-mortem dissection showed that generally the proportion of the sexes in each tube was three females to five males.

Preoviposition period. Beetles confined immediately after collection in the field did not lay eggs for some considerable time. Thus in 1939 beetles collected on 12 Apr. had a preoviposition period varying from 28 to 41 days with an average of 37 days, while those collected between 9 and 11 May had a preoviposition period of 13–15 days. In 1941 beetles collected on 2 May had a preoviposition period of 38 days while those collected on 29 May laid after 7 days. In 1939 only a single oviposition was noted before 11 May, while in 1941 the first oviposition occurred on 27 May. During the preoviposition period the

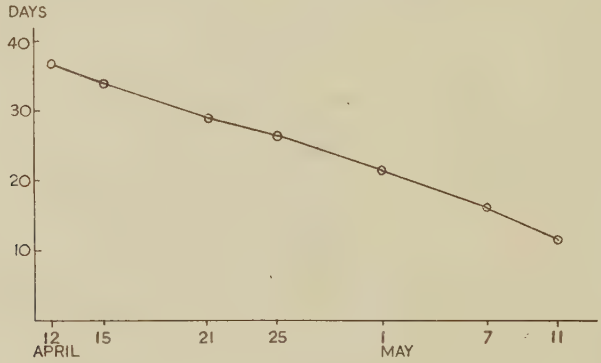

Fig. 6. Preoviposition period of *A. obscurus* L. in relation to date of collection of beetles in the field in 1939.

females become mature and ripe for egg laying. Fig. 6 shows the length of preoviposition period in relation to date of collection in the field.

Oviposition was observed many times. The female extruded the ovipositor and pushed it into the soil. In all cases some eggs were inserted in the soil close to the wall of the tube and could be seen through the glass, but most eggs were deposited in the centre of the soil surface in the tube. Thus in ten tubes the numbers of eggs inserted close to the wall were 6, 30, 6, 11, 21, 31, 8, 19, 21, 6, while the number of eggs deposited in the central areas of the soil surfaces were 92, 34, 30, 236, 37, 71, 64, 98, 80, and 62 respectively.

Depth of oviposition. In no instance were the eggs inserted deeply into the soil. Sometimes they were deposited about $\frac{1}{2}$ in. below the surface, but usually they were found at depths of $\frac{1}{8}$–$\frac{3}{16}$ in. below the surface. In a few instances the beetles burrowed down into the soil close to the wall of the tube, but the burrows rarely extended for more than 1 in. below the surface.

In these tubes, and in the experiments made by other workers, the beetles had the most favourable conditions for oviposition. The soil was not strongly compacted and was uniformly damp. The atmosphere above the soil was moist and there was no chance of drying out. The temperature too was generally higher than in the field and the diurnal variations

were less. In view of the ready oviposition the conditions in the tubes were evidently preferential for the beetles. In the field all the factors enumerated above vary considerably. It is therefore probable that in the absence of such conditions on the immediate surface of the soil the beetles seek cracks and crevices in the soil and that the eggs are inserted deeper in the soil. In support of this, attempts to induce beetles to oviposit in tubes containing dry soil, taken from the surface of an experimental plot failed, the females retaining their eggs although the oviducts were closely packed. On transference to tubes containing moist soil from 2 in. below the surface of the same plot the beetles deposited eggs, oviposition occurring in some cases within minutes of the transference. Gough and Evans (unpublished MS.) in an experiment to test the ovipositional preferences of the adults in 1939 found that the soil in boxes kept in a glasshouse had retreated from the sides of each box leaving a wide crack round the perimeter. In only three out of twenty-three boxes were eggs found in the central area, ten eggs only being recovered from this site in the three boxes. From the soil about the walls of the boxes 795 eggs were obtained, indicating that the beetles had descended the marginal crevices to deposit eggs. An analysis of the data obtained by Gough and Evans showed that even though eggs were found in the layer of soil 5–7½ in. below the surface 67 % were deposited in the top 2½ in. of soil. Salt and Hollick (unpublished work) gave details of the population of wireworms in per sq. ft. of soil examined in 6 in. layers. In a sample taken 1 Mar. 1941 analysis of their figures which were obtained by a highly accurate technique showed that 72 % of the larvae less than 4·1 mm. in length (larvae which were not more than 9 months old) were found in the top 6 in. of soil. Since Miles & Cohen (1941) have shown that the larvae of *A. obscurus* reach a length of 4·5–5·5 mm. 4–5 months after hatching, it follows that the small larvae found by Salt and Hollick had hatched from eggs laid the previous year. Such young larvae are not very active and it is permissible to assume that they had not moved greatly from the sites in which they hatched.

The available evidence therefore indicates that while under laboratory conditions the beetles lay eggs in the immediate surface soil, in the field they may deposit eggs in the deeper layers but that oviposition probably occurs mainly in the top 6 in. and it is likely that most eggs are laid in the top 2–3 in. of soil. Moreover, the structure of the insects is not of a fossorial type, and supports the above conclusion.

Preferred habitat for oviposition. While the beetles may deposit eggs in cracks and crevices in the soil, the mechanical condition of the soil during the oviposition period is not the controlling factor. The highest populations of wireworms occur in grassland. Thus in Romney Marsh, where some of the highest numbers are recorded, rye grass and white clover constitute from a third to nearly nine-tenths of the herbage (Tansley, 1939). In Cheshire the highest populations have been recorded in an almost completely grassland (pasture) district. Under such conditions deep cracks would not generally occur in the soil and there would be less variation in temperature of the surface soil, and a higher relative humidity of the atmosphere immediately over the soil than would occur on fallow land or land with a low plant population, e.g. cereals and root crops. Guéniat (1934) suggested that the presence of clover is a characteristic of the sites chosen for oviposition. This is not so, however, since high populations have been recorded from grassland where clovers were almost completely absent. At Warburton the herbage on one of the grass plots is a pure culture of couch grass, yet in the period 1938–41 the population increased

considerably, and sampling in December 1941 showed that over 52% of the wireworms were not more than 19 months old so that oviposition must have occurred freely in 1940 and 1941. Gough and Evans (unpublished MS.) in their experiments recovered most eggs from grass plots and least from fallow plots, while the number of eggs recovered from pure clover plots was significantly lower than the number obtained from the grass plots.

These observations coupled with the information concerning the activity of the beetles on the surface show that grassland is the preferred habitat of the insect for oviposition.

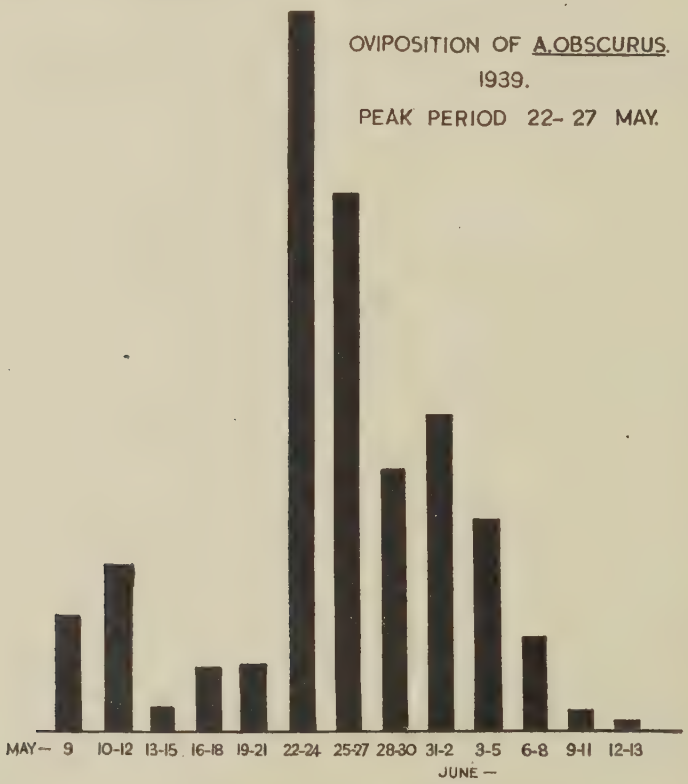

Fig. 7. Peak period of oviposition of *A. obscurus* L. in 1939.

The egg and the size of the egg mass. The egg of *A. obscurus* is translucent white and ovo-spherical. There is no sign of an external micropyle and the chorion is not sculptured, the surface being smooth and glistening in appearance and somewhat adhesive. The chorion is not firm and the egg is often deformed out of the regular ovo-spherical shape. This deformation is often seen when several eggs cohere and is responsible for some variation in size. The average size of the egg is 0·61 × 0·53 mm., and the range in size extends from 0·52 × 0·48 mm. to 0·65 × 0·53 mm.

The number of eggs laid in one spot varied considerably. Single eggs were often found. In 1938 and 1939 egg masses of one to twelve were frequent while sometimes considerable numbers were laid close together though unattached. Sampling in the late autumn and

winter sometimes leads to finding a large number of larvae of the current year's generation in a small volume of soil. In March 1940, twenty-six wireworms measuring less than 7·0 mm. were found close together and in 1941 on several occasions ten to fourteen small wireworms were found in very close proximity.

Peak period of oviposition. In 1938 the first eggs were obtained in the laboratory on 14 May. In 1939 oviposition commenced on 9 May and in 1941 on 12 May. Beetles that had overwintered in the laboratory did not oviposit earlier than those collected in the field

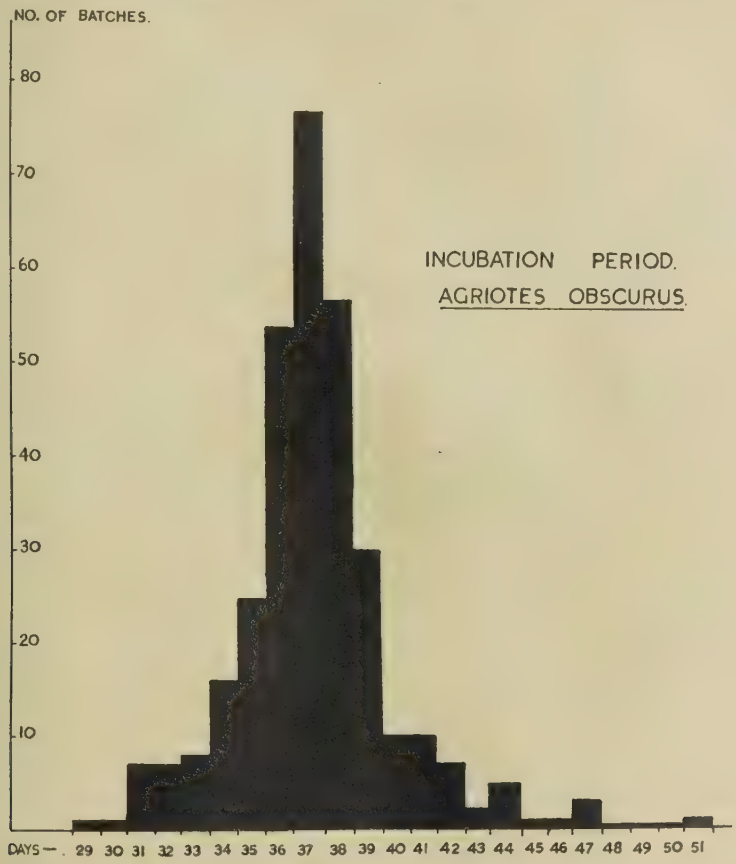

Fig. 8. Frequency of incubation period of *A. obscurus* L. in 1939.

in the late spring. These dates are much earlier (2–4 weeks) than those recorded by Guéniat (1934), with the exception of his first record for 1933 which was 16 May. Langenbuch (1932) gave 26 June as the date of the first oviposition while Zolk (1924), Chrzanowski (1927) and Subklew (1934) gave 16 June, 29 May and 14–25 June respectively.

Oviposition was last observed on 6 June in 1938, 14 June in 1939, and 26 June in 1941; the period of oviposition does not therefore last much longer than 4–5 weeks under laboratory conditions.

By examining each oviposition tube daily and transferring the beetles to a fresh tube of

soil as soon as eggs were noted, it was possible to ascertain the number of eggs laid by the females and to decide when the peak period of oviposition occurred. 323 tubes were thus obtained in 1939, containing between 5000 and 6000 eggs. Fig. 7 shows that in 1939 the peak period for oviposition occurred from 22 to 27 May. In the three-day period 22–24 May 1542 eggs were deposited, and in the period 25–27 May 1154 eggs were counted.

10. Incubation period

In 1938, the first and last dates on which eggs hatched were 6 and 17 July; the corresponding dates in 1939 were 18 June and 20 July. As the season progressed the eggs required a shorter incubation period. Thus in 1938 eggs laid in the five days 16–20 May required an average incubation period of 59 days, while those deposited from 5 to 9 June required 40 days. In 1939 incubation periods were generally shorter. Eggs laid from 9 to 13 May hatched in 43 days, while those laid from 24 to 28 June required only 36 days. The frequency of incubation period in this year is shown in Fig. 8.

The incubation period for the eggs of *A. obscurus* has been given by various authors and the range is from 25 to 44 days. Probably this range is due to variation in the dates of oviposition of the eggs on which the observations were made.

11. Number of eggs laid by the female

There is considerable variation in the estimates of the number of eggs laid by a single female. Thus Horst (1922) estimated 200–300 eggs per female, Chrzanowski (1927) 112–127, Langenbuch (1932) 119 and Subklew (1934) 217–237 eggs per female. In 1939 several series of tubes were examined to estimate the total number of eggs laid by a known number of females. The results are given in Table 5, which shows that the average number of eggs per female in the ten series varied from 70 to 186. The total number of females was twenty-two and the total number of eggs laid was 2262, giving an average for the whole series of 103 eggs per female.

Table 5. *Number of eggs laid by* Agriotes obscurus

Series	No. of eggs laid	No. of females	Av. no. eggs per female
1	186	1	186
2	132	1	132
3	248	2	124
4	327	3	109
5	313	3	104
6	403	4	101
7	98	1	98
8	167	2	83
9	247	3	82
10	141	2	70
Total	2262	22	103

12. Mortality of the eggs during incubation

In a few instances, egg masses were destroyed by moulds before hatching, but generally the eggs were remarkably resistant to soil fungi although the soil used in the tubes was taken directly from the field, the only treatment used being sifting to remove stones.

Discussion

In the north-west of England the dominant species of Elaterid is *Agriotes obscurus* L. The habits of the insect are influenced greatly by vegetation and atmospheric conditions. Probably the essential factor involved is a high plant population, which offers close cover, and because of transpiration also offers a high interplant air humidity. While the type of herbage may affect the activity of the insect and influence oviposition, it would appear that the plant constituents of good grassland, clovers and grasses of high agricultural value, are not necessarily more conducive to oviposition than the herbage of grassland of low agricultural value. It is also doubtful whether the factors of soil texture, *p*H and available plant foods, have any consistent effects on the oviposition preferences of the insects.

Weather conditions, particularly the moisture factor, are important during the oviposition season, and long-term observations of weather, coupled with trapping, may afford a means of forecasting the likelihood of seasons of intense oviposition and of subsequent high infestation of the soil. Moisture is important in influencing both the habits of the beetles and the incubation of the eggs, and also in affecting the survival of the subsequent larvae. Exposure of larvae to the free atmosphere or their confinement in soil or artificial solid media of low water content results in the rapid death of the insects after a period of great activity. This activity is connected with efforts to leave the dry habitat and find a moist one. Thus it seems that soils with relatively high water holding capacities which do not dry out offer the best conditions for the larvae, and especially for the occurrence of high populations. Again long-term observation of weather and regional classification of soils may produce information concerning the chances of survival of the larvae which may supplement the information concerning the adults.

Summary

The distribution of *Agriotes obscurus* in the north-west of England is outlined. The habits of the adult are described and vegetation and weather are shown to be important in influencing the activity and oviposition of the insects. The periods of oviposition and incubation for *A. obscurus* are given. It is suggested that long term observation of the habits of the adult insects in relation to weather, and similar observations concerning relations between soil type, water holding capacity and density of infestation of the larvae, may afford means of forecasting the incidence and maintenance of heavy infestations of wireworms.

The writer is indebted to Dr H. W. Miles under whose direction the work has been done. He has enabled the author to obtain continuity of observation and to make use of the records accumulated in advisory sampling for wireworms. Dr R. Stewart has been helpful in discussing certain phases of the work and Mr V. E. Henderson has given valued assistance in dealing with numerical data.

REFERENCES

ADRIANOV, A. P. (1914). Report on the work of the Entomological Bureau (of Kaluga) in 1913–14. Abstr. in *Rev. appl. Ent.* A, **3**, 309.

CHRZANOWSKI, A. (1927). Pewne dane z biologji i ekologji niektórych Elateridae (*Agriotes obscurus*) i nowe metody ich zwalczania. Warszawa (with a résumé in German), pp. 1–52, quoted in Guéniat, E. (1934).

FRYER, J. C. F. (1941). Time of flight of beetles of the genus *Agriotes*. *Ent. mon. Mag.* **77**, 280.

GUÉNIAT, E. (1934). Contribution à l'étude du développement et de la morphologie de quelques Elatérides (Coléoptères). *Mitt. Schweiz. ent. Ges.* **16**, 167–298.

HAWKINS, J. H. (1936). The bionomics and control of wireworms in Maine. *Bull. Me. agric. exp. Sta.* no. 381.

HORST, A. (1922). Untersuchungen über *Agriotes obscurus* L. Ein beitrag zur kenntnis der biologie und morphologie der elateriden und ihrer larven. *Arch. Naturgesch.* **88**, 1–90.

LANGENBUCH, R. (1932). Beiträge zur kenntnis der biologie von *Agriotes lineatus* L. und *Agriotes obscurus* L. *Z. angew. Ent.* **19**, 278.

MESNIL, L. (1930). Nos connaissances actuelles sur les Elatérides nuisibles en France. *Rev. Path. vég.* **17**, 178.

MILES, H. W. & COHEN, M. (1938). Investigations on wireworms and their control. 1st Rep. *J.R. Lancs. agric. Soc.* pp. 58–79.

—— —— (1941). Investigations on wireworms and their control. 3rd Rep. *J.R. Lancs. agric. Soc.* pp. 31–58.

NEUWEILER, E. (1926). Beobachtungen über die drahtwürmer. *Landw. Jb. Schweiz.* **40**, 135–42.

POSPELOVA, V. M. (1939). Notes on the wireworms in the region of Tomsk [in Russian], with a summary in English. *Plant Prot.* **18**, 16–35. Abstr. in *Rev. appl. Ent.* A, **27**, 628–9.

REGNIER, R. (1928). Les Taupins nuisibles en grande culture. Contribution à l'étude de l'*A. obscurus* L. *Rev. Path. vég.* **15**, 40.

RYMER ROBERTS, A. W. (1919). On the life history of wireworms of the genus *Agriotes* Esch. with notes on that of *Athous haemorrhoidalis* F. Part I. *Ann. appl. Biol.* **6**, 116.

SHARP, W. E. (1908). *Coleoptera of Lancashire and Cheshire*, p. 53.

STEINER, L. F. (1929). Codling moth bait studies. *J. econ. Ent.* **22**, 648.

STOCKER, O. (1923). Klimamessungen auf kleinstem raum an wiesen-, wald- und heidepflanzen. *Ber. dtsch. bot. Ges.* **41**, 145.

STRICKLAND, E. H. (1933). The biology of prairie inhabiting wireworms. *Proc. World's Grain Exhibition and Conference, Canada*, **2**, 520.

SUBKLEW, W. (1934). *Agriotes lineatus* L. und *A. obscurus* L. (Ein beitrag zu ihrer morphologie und biologie.) *Z. angew. Ent.* **21**, 96.

TANSLEY, A. G. (1939). *The British Islands and their Vegetation*, p. 575. Cambridge.

ZOLK, K. (1924). Mönda uuemat tumeda Wiljanaksuri (*Agriotes obscurus*) bioloogiast. (Some new data on the biology of *A. obscurus*.) *Tartu Ülikooli Entomoloogia-katsejaama teadaanded*, no. 4, Tartu. Abstr. in *Rev. appl. Ent.* A, **13**, 67.

(*Received* 10 *October* 1941)

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

ORDINARY MEETING of the Association of Applied Biologists held in the London School of Hygiene and Tropical Medicine, on Friday, 5 December 1941, at 11 a.m., the Chair being occupied by the President, Dr Hubert Martin.

A. *Discussion on some problems in war-time horticulture with particular reference to vegetable production*

The following papers were read:

Problems in vegetable production: introductory. By H. V. TAYLOR, D.Sc.

Some problems of the glasshouse industry in war time. By W. F. BEWLEY, C.B.E., D.Sc.

Some aspects of the nutrition of vegetable crops. By T. WALLACE, D.Sc.

The control of weeds in onions. By G. E. BLACKMAN, M.A.

B. *Some aspects of tree surgery in Canada.* By CHESTER COOK (Bannatyne Tree Service, Toronto)

PROBLEMS IN VEGETABLE PRODUCTION: INTRODUCTORY

By H. V. TAYLOR, D.Sc.
Ministry of Agriculture and Fisheries

The rise of vegetables. The recognition of the fact that vitamins and minerals were important to the human system created a very different attitude to vegetables and particularly as to the part they should play during the war period. Investigations of the problem showed that in the main vegetables were a cheap source of vitamins and could be home produced and, in consequence, could take the place of the expensive imported fruits that supplied the public with the principal part of its vitamin C during peace time. Fruit imports had to cease and the vegetable area had to be expanded to make good the deficiency. The wise and very learned nutritionalists tried to assure the public that 'Fruit was not an essential article of diet and that vegetables could supply all the necessary vitamins and minerals.' Whether the public believed this doctrine is doubtful, but it is true that the vegetables have gained in importance and are now eaten in greater volume.

Making good the loss of imports. The war machine not only caused a reduction in fruit imports but virtually stopped the imports of vegetables which formerly came from the Channel Islands, from Holland, France, Italy and the Mediterranean regions. The chief supplies cut off were onions—and nearly 95 % of our requirements were imported in peace time—tomatoes, dried peas and carrots.

The problem with vegetables was to secure an increased production of these kinds and simultaneously to secure an expansion of many other kinds for which the demand had increased during war time. Vegetables such as beetroot, parsnip and swedes though only needed in limited quantities normally, are sought in greatly increased amounts during the war period. Perhaps the sugar contents are acceptable to a human system kept short of sugar supplies.

A few kinds of vegetables, of which asparagus and celery are the typical examples, have been weighed in the vitamin balance and found wanting. Costly to buy and delicious to eat these vegetables stand condemned as imposters with the official hand raised against them! The area they formerly occupied will be filled with more deserving subjects.

Seed supplies. The first major problem concerned the supplies of seeds and the seeds of many kinds—onions, carrots, leeks, beans, peas, cauliflowers—were formerly imported. Those who are on

the look out for pests may note that the sources from which the seed has been obtained have been changed from Holland, Hungary and North Africa to the U.S.A., Egypt and Australia. One would expect the seed from these new sources to be associated with new kinds of pests and diseases. These may turn up in the future.

One problem has already presented itself and that concerns the onion seed from America. The seed seems good and healthy; it germinates well and produces good plants. The bulbs begin to swell, but when they reach the size of walnuts ripening sets in and the crop is finished prematurely. The yield is barely 3 tons/acre as against more than double that raised from European seed. This is as disappointing as it is disturbing for it would seem that for a time this country will be compelled to rely on America for onion seed. The author will not venture to theorise as to the cause of this, but leave that to the more fertile imagination of the readers.

Problems. Onions. As mentioned previously the public relied on the imported onion in peace time and this was the first crop chosen for vast expansion. This presented many problems and the one concerning seed has been mentioned. The second one concerned onion setts. Onion setts as we know them are very small onions imported from Greece and Eastern Europe. When planted the small onion increases in size from $\frac{1}{2}$ in. diam. to about 2 in. Gardeners in the north-west and in Scotland have more success with these than with seed. These small onion setts are said to have been specially grown and raised before being ripe and stored in such conditions that development of the flower bud is arrested. This is a very interesting problem for it is our experience that the small onions from our garden crops will just run to seed if planted. It is thus important to discover, 'When is a small onion an onion sett?' and this problem is now the subject of research by Prof. Gregory and Mr Heath of the Imperial College of Science and by Dr Tincker and Mr Brown of the R.H.S. Laboratories of Wisley.

The onion plant produces little foliage which is quite unable to battle with weeds so that onion fields require much hand weeding, and it seemed improbable that sufficient labour could be spared during war time to hand weed the vastly expanded acreage. The problem was to find some chemical spray noxious to the weeds but not harmful to the onions and this task was given to Mr G. Blackman to solve and no doubt he will give an account of his experiments and successes. If the results are fully successful the production of onions will become a much simpler task.

The further problem with onions was caused by adverse weather. It is said that the onion plant does best when the soil is warm and full of moisture during the early and middle stages of growth, and then bone dry in August to ripen the bulb. The weather during 1941 was the opposite to this with the result that by August and September a large proportion of the crop had swollen necks and were not good bulbs. These swollen necks enjoy many names, e.g. 'romps' in Bedfordshire, 'pipers' in Worcestershire and 'bull necks' elsewhere. At any rate these 'bull-necked' onions presented a problem for the legal people. If they were bulb onions the growers should hand the crop to the National Vegetable Marketing Co. for distribution, but if not, the growers were free to sell anywhere. I believe the law was disregarded and the bull necks sold as salad onions to everyone's great satisfaction!

Carrots. Our old equestrian friend, the donkey, long ago demonstrated that the carrot was the vegetable par excellence. In more recent times the medical profession has acclaimed its virtue and stated with confidence that the public during the war period should double the consumption of carrots. This meant doubling the acreage. The growers feared that the carrot fly would destroy their effort but happily this did not prove to be the case, for the 1941 carrot area was really large and the crop very weighty. There was one problem—a large proportion, often 25% of the carrots were cracked. This is said to be due to heavy rains following the drought period. There may be contributory causes and these need thought and study. Would more humus in the soil prevent cracking or otherwise? What are the effects of dressings of nitrogen and of potash?—would these have increased or decreased cracking? At present there is no data either way, but it is hoped some experiments will supply evidence on this important economic problem.

Leeks. The problem here is one of seed shortage. The leek flowers come late in the summer and even with luck the seeds are not ready until late October. In wet summers, as in 1941, most of the seed is lost and shortage prevails. Can scientists not devise some methods of treatment of the seed that would stimulate the plant to earlier flowering and seeding. It should prove an interesting problem for a plant physiologist and let us hope lead to the production of more leek seed.

Brassica. There were two outstanding troubles this year—the flea beetle which is controllable by powdering with pyrethrum, and club root an old and persistent disease which has reappeared almost

everywhere during the wet summer of 1941. It weakens the winter brassica plants and has reduced the winter food. This is a problem in itself, but there is another, for at present waste vegetable material is put into the compost heap to rot down. What happens to the club root and to the fungus diseases in the compost heap? Are the diseases destroyed by the heat of the compost or incubated and increased? Here again the scientific knowledge is slender and definite facts are lacking.

Dried peas and haricot beans. The protein-rich vegetables, peas and beans, were formerly imported in large quantities and are now in reduced supply. There has been a large increase in the area laid down to peas, to supply dried peas, and to French beans to provide haricots. During 1941 the harvesting has not been very successful and the sample has been stained and discoloured, with some losses. When harvested the haulm has been too green and wet and disease (probably *Botrytis* sp.) has appeared to attack the pods and discolour the seeds. Is it not possible to spray the material with some fungicide as a preventative? This is a war time problem well worthy of study.

If there was time the problems of other crops could be enumerated.

Conclusions. It is realized that listeners have a right to expect that a paper should provide some knowledge and therefore apologies are due that this has not been done. The unsolved problems have been exposed and the lack of knowledge laid bare. It is hoped, however, that this exposure may stimulate others to tackle the problems and so make good some of the deficiencies in our knowledge. If this happens the paper will have been worth while.

SOME PROBLEMS OF THE GLASSHOUSE INDUSTRY IN WAR TIME

By W. F. Bewley, C.B.E., D.Sc.

Experimental and Research Station, Cheshunt, Herts

Apart from certain difficulties in obtaining supplies the chief problem is how best to satisfy the demand of the Government for a very greatly increased production of tomatoes. This involves: (*a*) increasing the yield per acre on nurseries devoted to tomatoes in peace time; (*b*) growing the heaviest crop possible in houses previously devoted to flower crops; (*c*) increasing production under grapes, peaches, and nectarines; (*d*) growing a crop in small, low houses usually devoted to cucumbers, ferns, pot plants and bedding stuff; (*e*) growing plants in houses where soil has been used merely as a standing-out ground for plants in boxes, pots, etc., and where the soil is 'sick'; (*f*) finding substitutes for manures and fertilizers which are on short supply.

I propose to deal only with the last two owing to the limited time at my disposal and because they are the most suitable for discussion at a meeting of this kind.

I. *Soils which have formed the floor of glasshouses which have not been cultivated for some time and which are unsuitable for tomatoes without special treatment*

The problem presented by such soils is not an unusual one for it bears some resemblance to that associated with the soil under cucumber beds and those under mushroom beds where these crops are grown year after year without change. Samples from these plant houses have several points in common as follows: (*a*) they are surfaced by a tightly compressed crust, usually covered with a heavy algal growth; (*b*) they have an unpleasant smell when first broken up; (*c*) the bacterial count is low—often less than 1,000,000 bacteria/g. and the proportion of anaerobes and spore formers is greater than in cultivated soil in adjoining houses; (*d*) they seem capable of absorbing large volumes of oxygen.

These harmful soils have usually remained undisturbed for many years. Some are very wet down to a depth of 18 in., others are wet near the surface (top 2–3 in.) and are then very dry underneath.

The scientific investigation of these soils is in progress, and will no doubt be prolonged. Fortunately, however, a practical solution based on scientific principles was not difficult to devise. It involves treatment of the soil to increase bacterial activity and produce a balanced population, followed by sterilization. Ideally the treatment should commence not later than the beginning of October. The soil is forked over breaking up all lumps. If it is dry sufficient water is applied to wet it down to a depth of 20–24 in. Hydrated lime at the rate of 8 oz. and superphosphate 4 oz./sq. yd., is spread uniformly over the surface and then the soil is thrown up into steep ridges about 18 in. high and 18 in. wide at the base, mixing the lime and superphosphate with it as uniformly as possible.

Six to eight weeks later the ground is levelled and sterilized either by steaming or with formaldehyde and then wheat or oat straw is incorporated in the soil as almost vertical walls.

II. *War time fertilizer treatment*

(*a*) *Horse manure.* Only the cleanest horse manure containing straw is used for glasshouse work in peace time. To-day supplies are limited and substitutes must be used. One of the best consists of straw decomposed to the required degree by the A.D.C.O. process. Such material has proved useful in glasshouse work since its discovery some 24 years ago. Clean compost prepared from selected tomato shoots and leaves is also being used on an increasing scale. Coarse grasses and nettles, the latter being particularly rich in potash, also make useful compost, particularly if applied before steaming which kills weed seeds. Bracken and seaweed are also useful in districts where they are available.

(*b*) *Potash.* Sulphate of potash has been reserved for tomatoes under glass and is rationed at 8 cwt./acre. It must be supplied to the grower unmixed with other fertilizers unless he asks specially for it to be delivered in a compounded fertilizer. Muriate of potash cannot be purchased for tomatoes under glass. Substitutes which are available are flue and cement dusts, some of which are good sources of potash, and the so-called 'organic' potash. Potash salts should always be regarded with suspicion until the sample concerned has been tested on plants and proved harmless, for some samples have proved to be injurious to the tomato.

(*c*) *Plant ash.* Ash containing fair amounts of potassium carbonate can be prepared by burning grass, young bracken, hedge clippings and weeds, especially nettles, and growers are urged to make as much as they can during the summer. The material must be reasonably dry when ready for burning and the ash must be stored in a dry place.

During the short time at my disposal I have tried to indicate some of the glasshouse growers' war time difficulties. I would add, however, that in my opinion they shrink into insignificance in comparison with a problem which hovers perhaps nearer than any of us think—the problem of saving the industry when peace comes and our markets are flooded with produce from abroad. It is a problem which should make us furiously to think.

SOME ASPECTS OF THE NUTRITION OF VEGETABLE CROPS

By T. Wallace, D.Sc.

Long Ashton Research Station, Bristol

At the outset, I should like to make the point that in this country problems of the nutrition of vegetable crops have not been investigated by scientific workers to anything like the same extent as those of agricultural and fruit crops. For this reason our knowledge of the former is less extensive and in practice vegetable growers must still rely to a large extent on experience. This position is unfortunate under present conditions, when vegetable production is of such vital importance to the National effort and when vegetable growers are faced with new and difficult problems, due to restricted supplies of the manures and fertilizers on which they relied in peace time. In the present paper I shall endeavour to focus attention on some aspects of the problems which confront the practical grower and to bring before you points of importance in present-day problems.

Before discussing these points, I should like you to bear in mind the fact that vegetable crops may be grown under two conditions of culture: (*a*) in ordinary farming rotations, where the land is not specially treated, though it must be in good heart; and (*b*) under conditions requiring a specially high level of soil fertility and special manurial and cultural treatments, for intensive cropping and the production of special crops. It is particularly in connexion with conditions under (*b*) that many of the special nutritional problems of vegetable culture arise and to which the following points mainly refer.

The building up and maintenance of soil fertility for intensive vegetable production

For intensive vegetable production the condition of the soil must be suitable for a range of crops to follow in continuous succession, without breaks in supplies even under unfavourable weather conditions. The 'timing' of crops is of great importance, gluts must be avoided and cropping periods must be lengthened by the production of 'out of season' crops.

To achieve these ends it is necessary to build up soil fertility to a very high level; in short, the 'vegetable soil' must be 'made', and must be regarded as a highly artificial soil requiring special measures for the maintenance of its special properties. Much can be done to ensure success in the initial choice of the site and soil. The soil should be naturally of a friable nature, of good depth, well drained in the surface layers but not highly susceptible to drought, and a fairly high water table may be advantageous.

In the 'made' soil the following conditions are essential: (1) there must be a good depth of soil and there must be no 'pan', due either to 'iron pan' or mechanical packing of the soil; (2) drainage must not be defective at any season of the year; (3) soil reaction should be slightly above *p*H 6·0 and a small reserve of carbonate of lime is generally desirable; (4) there must be a plentiful supply of organic matter, some of which should be in a state of active decomposition; and (5) plant nutrients, especially nitrogen, phosphates and potash, must be present in adequate amounts.

Lime content and liming

The correct use of lime is fundamental in the building up and maintenance of suitable soil conditions for vegetable culture. Lime is a basic factor in tilth formation, and together with organic matter, ensures suitable conditions for seed bed formation, root development and water movement within the soil. It is also of great importance as a plant food for vegetable crops, since many of the crops grown, especially the leafy brassicae, feed heavily on lime. Vanstone & Knapman (1939), for example, have shown that a good broccoli crop may remove as much as 110 lb. CaO per acre from the soil. In addition to the need of vegetable crops for lime, it should be remembered that the losses of lime through drainage waters from such highly cultivated soils are likely to be much greater than under farming rotations. The dangers attending the growing of brassica crops on lime deficient soils, due to club root disease, needs no stressing here and brassicae play a dominant role in most vegetable growing enterprises. Lime, however, should not be used indiscriminately in intensive vegetable culture, since the types of soils used and the kinds of crops grown may be injured by excessive liming. In particular, deficiencies of manganese and boron may be induced by over-liming (see Davies, 1939; Wallace, 1940; Wolf, 1940).

Organic matter

The importance of maintaining a high content of organic matter, especially organic matter in the process of active decomposition, in soils used for intensive vegetable culture cannot be overstressed. Practical growers lay great emphasis on the organic matter status of their soils and its maintenance is a problem which is ever before them. As previously stated organic matter, together with lime, is fundamental to good tilth and suitable water-relationships in soils. It also supplies the source of energy for circulating many of the soil nutrients and acts as a regulator and storehouse of plant food supplies. Organic materials contribute considerable amounts of the major plant foods to the soil and may also add sufficient quantities of the so-called minor elements to make good deficiencies of these. At high *p*H values, above 6·6, organic matter appears capable of rendering manganese unavailable to several vegetable crops. It should be noted that because of the intensive methods of cultivation employed in vegetable culture the rate of organic matter decomposition is very high.

If adequate supplies of good quality stable manure were available present-day problems of vegetable growing would be greatly simplified, but supplies are very short and in some districts are not available at all. Various materials and methods are used to maintain the organic matter content of soils under vegetable culture. Sewage sludge, composts, treated town refuse, screened dusts and a variety of other waste materials are used whilst green cropping is also practised in some cases. Where vegetable cropping is practised with general farming, organic matter depletion may be remedied by interspersing leys in the rotation.

The use of waste materials for supplying organic matter is worthy of investigation, since some of these are available in very large quantities and their use would greatly benefit the community in other ways. One important point met with in using a town waste material for vegetable culture has been that the material may cause a temporary deficiency in nitrogen, due presumably to an unfavourable carbohydrate/nitrogen ratio. In fact, many of these materials should be regarded as supplying organic matter rather than of adding large amounts of N, P, and K, and should be supplemented by liberal dressings of fertilizers. Waste materials should be tested for lime content before using them extensively as this may be high. Some town waste products have been shown to contain appreciable amounts of minor element nutrients.

Under present conditions of potash shortage the method of utilizing vegetable residues by direct ploughing-in, after treatment with a nitrogenous fertilizer, should be compared with composting procedures, since the former precludes losses of minerals which may occur during composting.

Nutrient elements

As in the case of farm crops, the main emphasis must be laid on problems relating to nitrogen, phosphates and potash, but other nutrients such as calcium, magnesium, iron, manganese, boron, sodium and chlorine present problems of practical importance, some of which are illustrated here. The large requirements of brassicae for major nutrients are shown in the results of Vanstone & Knapman (1939) for broccoli crops grown in Devon and Cornwall, where the following average amounts per acre were removed from the soil: N 204 lb.; P_2O_5 70 lb.; K_2O 240 lb.; CaO 110 lb. These compare with values for wheat grain and straw quoted by Vanstone & Knapman from Comber: N 60 lb.; P_2O_5 20 lb.; K_2O 30 lb.; CaO 10 lb. Lewis (1938) also estimated that in his experiments a crop of Brussels sprouts removed 300 lb. N/acre. Results such as these for many kinds of vegetable crops emphasize, in particular, the very large requirements of vegetable crops for nitrogen and potash, this great need for potash often not being realized by growers and technical officers who have been used to farm crops.

(*a*) *Nitrogen, phosphates and potash.* Vegetable growers are fully aware of the importance of the liberal application of nitrogen to their crops, but opinion is often divided on the relative merits of different forms of nitrogen for use on various crops and a sharp distinction is drawn between organic and inorganic fertilizers. This last point is of some importance at present since supplies of 'organic' nitrogenous materials are very short and may be curtailed still further during the period of the war. Even if growers must rely wholly on inorganic sources of nitrogen, mainly sulphate of ammonia, there is no reason why success should not be achieved with most crops. Salad crops, such as lettuce, and the 'timing' of crops may present difficulties.

Vegetable growers invariably use phosphates very liberally and old market garden soils show very large amounts of 'available' phosphate. Whether such liberal use is sound practice requires investigation and possibly economies in the rates of dressings could be made without sacrificing efficiency. During the war supplies of phosphates are likely to be mainly in the form of concentrated soluble phosphates—superphosphate and triple superphosphate. These are eminently suited to vegetable production.

Imported potash may not be allotted for vegetable crops in 1942,* with the exception of potatoes, onions and carrots, and only home-produced waste products, such as flue dusts and wood ashes etc., will then be available. These latter should generally be suitable for vegetables if applied some time before sowing seeds. If they cannot be obtained the only courses open will be to endeavour to obtain some stable manure for use on crops with special potash needs, to conserve potash in all plant residues, to use salt on crops, such as beet, on which salt produces specific effects and appears to decrease the need for potash, and to avoid crops with high potash requirements on potash deficient soils. Other points requiring investigation in connexion with the use of potash manures for vegetables are the relative merits of sulphate and chloride materials, and the 'hardening' effects of potash in relation to such problems as succulence, frost injury, premature heading of cauliflowers, quality of produce, etc.

(*b*) *Other nutrient elements.* The main practical interests in elements other than nitrogen, potassium and phosphorus lie in deficiency effects of calcium, magnesium, iron, manganese and boron, and the effects of sodium and chlorine.

Calcium. In practice the problems of calcium deficiency are complicated by acidity effects. The deficiency has been noted on brassicae, causing imperfect development of the laminae of the leaves, resulting in strap-like effects and cupping. The practical remedy is liming.

Magnesium deficiency, in a mild form, is of common occurrence in brassica crops, but it has also been observed to cause crop failures. The deficiency occurs mainly on light sandy soils, especially those which are naturally acid. The most susceptible crops are cauliflowers and broccoli, but sprouts and kale and to a less extent savoys, cabbages and swedes may be affected. Several cases of the deficiency in potatoes and one in globe beet have been noted. Brassica crops are good indicators of the deficiency, as the symptoms shown are very characteristic: a chlorotic 'marbling' of the older foliage followed by the development of high yellow, red and purple tints. The remedy under war-

* Subsequent to reading this paper the Ministry of Agriculture has allocated ½ cwt. KCl per acre for market garden crops other than the priority crops for 1942.

time conditions must generally be a dressing of ground magnesian limestone of high magnesium content, e.g. 1 ton/acre. The burnt product is not recommended for horticultural work. Magnesium sulphate in various forms is also effective but supplies are very limited at present.

Iron deficiency may occur in crops such as spinach on highly calcareous soils and in such cases the best procedure will usually be to avoid susceptible crops.

Manganese deficiency is of fairly common occurrence in small areas but it is important to note that the deficiency can be easily induced in market garden soils by overliming. Two soil conditions which have always been associated with the deficiency in cases examined in this country, have been a high *p*H (above 6·5) and high organic matter. It seems that on some soils the combination of these renders the soil manganese unavailable to the plant.

Soil conditions under which the deficiency has been noted are as follows: (1) newly ploughed-out grassland on calcareous silts and clays; (2) reclaimed heath soils, subsequently overlimed; (3) thin peaty soils overlying calcareous subsoils, e.g. 'Skirt' soils of the Fens; (4) poorly-drained calcareous soils with high contents of organic matter; and (5) old market garden soils, built up with frequent dressings of stable manure and lime.

Deficiency effects have been noted in the following vegetable crops: globe beetroot (very susceptible), spinach and spinach beet, peas (marsh spot), parsnips, parsley, cos lettuce, potatoes, vegetable marrow, dwarf beans, runner beans and onions (last two very slight effects). Carrots appear to be resistant. The symptoms vary for different plants, but there is usually some form of chlorosis. Globe beetroot is the best indicator plant, for which the condition is similar to the well-known 'speckled yellows' of sugar beet.

Several control measures can be applied. Good quality dung is usually effective. Manganese sulphate or chloride can be applied as fertilizers (100 lb./acre), as dusts on the foliage of partly grown plants (20–60 lb./acre), or as sprays (5–20 lb./acre). The spray method is particularly suitable in market gardens, and for small areas an ordinary watering can be employed using about 5 gal./40 sq. yd. Sulphur, applied as a dust, at rates from 5 to 20 cwt./acre is very effective on slightly overlimed soils and acts by lowering the *p*H. This method may not be economic or practicable under war-time conditions.

Boron deficiency is known to occur on most brassicae, especially on cauliflower, and on swedes, turnips, beets and celery (Walker *et al.* 1941; Dennis & Dennis, 1939, 1940, 1941), but the writer has not observed the deficiency under market-garden conditions. The trouble usually occurs on light soils and can be aggravated by raising the *p*H (Wolf, 1940). The symptoms are well known. In brassicae, necrosis of the stem, dying of growing points, distortion and tinting of leaves: in swedes and turnips a browning of the flesh of the roots (brown heart), in beets, rotting of the roots (heart rot), scorching of foliage and death of growing points; and in celery the development of the 'cracked-stem' condition. Control can usually be effected by applying 20 lb./acre of granulated borax as a fertilizer.

Sodium. Although sodium is regarded as a non-essential element, certain crops such as beets respond markedly to salt and contain high amounts of sodium. In some cases salt may produce crop increases similar to those produced by muriate of potash. The point is of special importance in view of the present potash shortage.

Chlorine. Plants appear to be specific in respect of their behaviour towards chlorine. Some are injured and others appear to benefit. There is evidence in the case of tobacco that magnesium deficiency is less serious in the presence of KCl than when K_2SO_4 is used (Garner *et al.* 1923). At Long Ashton magnesium deficiency was marked on potatoes in 1941 where sulphate of potash was used but was not present where potash salts, containing KCl and NaCl, were given. Pending further evidence the result cannot be attributed with certainty to chloride since the sulphate plots had received heavier dressings of potash manures in previous years. The composition of the leaves of the potatoes from the two treatments was as follows:

Potash treatment	% dry matter				
	Ash	CaO	MgO	K_2O	P_2O_5
Sulphate	19·21	4·71	**0·17**	6·49	0·65
Potash salts	19·45	6·84	**0·71**	3·65	0·68

Points in the war-time manuring of various crops

Brassicae: cauliflowers, broccoli, kales, spring and summer cabbages, savoys, Brussels sprouts. These crops are all of great importance in war time and must continue to comprise the major part of

vegetable production. Provided land is in good heart and contains a reserve of lime, yields can be kept at a high level by liberal nitrogenous manuring. These crops can be grown very successfully on newly ploughed old grassland even when potash status is too low for many other kinds of vegetables.

Root crops: carrots, parsnips, beets, turnips, swedes. Carrots and parsnips, and to a less extent beets, seem to have higher requirements for potash than most vegetable crops (Dyer *et al.* 1913). Carrots are a priority crop for potash. Salt may be used to replace potash for beets and trials might also be made with carrots and parsnips. Beets should not be overlimed. Turnips and swedes succeed under fairly low potash conditions. Turnips need rich soil when grown for human consumption but swedes must not be overmanured if they are to be stored.

Legumes: peas, broad beans, runner beans, French beans. These crops should not be attempted on land deficient in potash and should be given a high priority by the grower for any potash supplies he may have.

Onions and leeks. Onions are a priority crop for potash and the crop appears to have a high potash requirement (Dyer *et al.* 1913). Onions seem generally to fail on newly ploughed grassland and it is not clear how far this is due to unfavourable potash conditions. Leeks should also be given some potash when possible and it is most important that they be given a priority by the grower for bulky organic materials.

Lettuce and spinach. Lettuce needs land in a high state of fertility. It often fails on newly ploughed-up grassland. Potash is important. Organic forms of nitrogen appear better than inorganic forms. Provided spinach is planted on land in good heart, the great need is for nitrogen.

REFERENCES

Davies, W. M. (1939). Manganese deficiency in relation to soils and crops. *Agric. Progr.* **16**, 45.

Dennis, A. C. & Dennis, R. W. G. (1939). Boron and plant life. Part III. Developments in agriculture and horticulture, 1937–8. *Fertil. Feed. St. J.* Reviews of Literature.

—— —— (1940–1). Boron and plant life. Part IV. Developments in agriculture and horticulture, 1939–40. *Fertil. Feed. St. J.* Reviews of Literature.

Dyer, B. & Shrivell, F. W. E. (1913). *The Manuring of Market Garden Crops.* London: Vinton and Co. Ltd.

Garner, W. W., McMurtrey, J. E., Bacon, C. W. & Moss, E. G. (1923). Sand drown, a chlorosis of tobacco. *J. agric. Res.* **23**, 27.

Lewis, A. H. (1938). The relative values of organic and inorganic nitrogen fertilisers. *Emp. J. exp. Agric.* **6**, 38.

Walker, J. C., McLean, J. G. & Jolivette, J. P. (1941). The boron deficiency disease in cabbage. *J. agric. Res.* **62**, 573.

Wallace, T. (1940). A note on manganese deficiency in agricultural and horticultural crops. *Ann. Rep., Long Ashton*, p. 19.

Wolf, B. (1940). Factors influencing availability of boron in soil and its distribution in plants. *Soil Sci.* **50**, 209.

Vanstone, E. & Knapman, C. E. H. (1939). On the quantities of nitrogen, phosphoric acid, potash and lime removed from the soil by a crop of Roscoff broccoli during its growth. *J. Pomol.* **17**, 85.

THE CONTROL OF WEEDS IN ONIONS

By G. E. Blackman, M.A.

Imperial College of Science and Technology, London, S.W. 7

During 1941 fourteen experiments were carried out on the chemical control of annual weeds in onion crops. Principal attention was paid to spraying with sulphuric acid solutions at rates of 120–160 gal./acre, in addition, however, sodium chlorate and dinitro-ortho-cresol were also tested. The experimental technique involved estimating the degree of control obtained by counting plant densities and determining the effects of the treatments on yield.

It was found that the concentration of sulphuric acid required to give a high degree of control is dependant upon the weed species. If spraying is carried out when the plants are still in the seedling

stage then field speedwell (*Veronica agrestis*), henbit dead nettle (*Lamium amplexicaule*) and penny cress (*Thlaspi arvensis*) can be eradicated by a 5·0% (vol./vol. conc. of pure acid) solution. For the destruction of chickweed (*Stellaria media*), groundsel (*Senecio vulgare*), fat hen (*Chenopodium album*), annual nettle (*Urtica urens*), shepherd's purse (*Capsella Bursa pastoris*), goose grass (*Galium aparine*), knot grass (*Polygonum aviculare*) and sow thistle (*Sonchus oleraceus*) it is necessary to employ a 7·5% concentration to kill these weeds in the early stages of growth.

The susceptibility of weeds to sulphuric acid is dependant upon the size and stage of development of the plants at the time of spraying. For knot grass and sow thistle it is essential to spray the plants while they are still young seedlings since their resistance to spraying increases rapidly with age. For fat hen, groundsel, annual nettle and shepherd's purse the change over from susceptibility to resistance is less clearly cut and therefore the period of spraying is less critical. In contrast, chickweed and speedwell can be successfully controlled even when in flower.

The effects of adding a wetting agent to the sulphuric acid are dependant upon the weed species and stage of development. For some weeds this addition brings about a greater control, for others there is no advantage. In a third group the incorporation of a wetting agent renders the spray less effective in the seedling stage and more effective in later stages, e.g. shepherd's purse.

Sodium chlorate at concentrations of 1–3% compares unfavourably with sulphuric acid for destroying annual weeds. Dinitro-ortho-cresol, which was only tested in one experiment, eradicated chickweed, fat hen, and annual nettle.

As against hand weeding, a single spraying with sulphuric acid increased the yield of onions in four out of eight experiments. In one experiment spraying decreased the yield; in no experiment did the addition of a wetting agent have a significant effect. In one out of the three experiments where the crop was twice sprayed with sulphuric acid the yield was higher than on the control. Sodium chlorate caused an increase in yield in two out of four experiments. Dinitro-ortho-cresol was found to be highly toxic to the onion.

From these results it is concluded that for the most effective control of weeds in onion crops it is necessary (i) to spray the seed bed just before the onion seedlings appear above ground, (ii) to spray the growing crop once or possibly twice in the season as soon as fresh weed seedlings appear. It is anticipated that in a normal year a total of two to three sprayings coupled with push hoeing will eliminate all hand weeding.

SOME ASPECTS OF TREE SURGERY IN CANADA

By Chester Cook

Royal Canadian Army Medical Corps

Twenty years ago little thought was given to the care of trees. When a tree became dangerous, owing to wind damage or other reasons, or when it became unsightly or unhealthy, it was removed. There was nothing else to be done because there was no one specialized in the care of trees. There is no excuse, however, for this negligence to-day. The tree surgeon has replaced the tree-butcher and the odd-job man. A glaring example of what the odd-job man can do was the case in a small Ontario town which was besieged by starlings and unemployed men. Because the starlings came to roost in the 80 ft. Silver Maples, someone got the brilliant idea of having the men on relief cut the trees to a level which might discourage the starlings. Four or five beautiful trees were hopelessly destroyed by slashing before the odd-job-man-foreman was replaced by a tree surgeon. It is more important to have a beautiful tree, even with the questionable inconvenience of starlings, than to have nothing but unsightly stumps. When the people were convinced of this, which it was not difficult to do with a few mutilated examples to look at, the necessary work was done for the trees by the most capable of the available men under close supervision.

A tree surgeon must be able to climb a tree without the aid of linesman's spurs, because spurs tear the bark, allowing easy entry for injurious organisms—insects, fungi, and bacteria. Instead he uses a 120 ft. rope, in one end of which is tied a double bowline of a size convenient to the worker. A small coil is made with the rope around the bowline, the last coil being drawn through. By holding the loop thus made it is possible to throw the rope to a height of about 30 ft. over a limb of the tree. The weight of the coil brings the rope within the climber's reach. If, after the rope is climbed to the first limb, it is found that the tree is still of too great a girth to shinny, the process is repeated.

When the top of the tree is reached, the bowline end of the rope is put around the tree and over one strong lateral branch. The climber then steps into the bowline, drawing it up to where he can sit in it comfortably, and ties the tautline (the 6 ft. end of rope left over after the bowline is tied) to the long part of the rope, i.e. the part reaching from the crotch to the ground. The knot used is on the principle of the clove hitch and known as the 'tautline hitch'. When the climber's weight is in his rope the knot will not slip until he loosens it temporarily by sliding it down the rope. When the knot is released it immediately tightens again.

The tools needed will be a pole-saw, hand-saw, and pole-pruners, which are tied to the rope by a ground man. The hand-saw when not in use is fastened to the climber's belt by a spring clip. The pole-saw is a curved blade, narrow at the tip, which allows it to be inserted in narrow crotches, and having the teeth on the inside and pointing towards the handle, which is an 8 ft. pole. The cut is made by the saw being drawn. If the teeth were pointed towards the tip the bark would be stripped on the last cut. On the handle, and on the opposite side to the saw, there is a hook which allows the saw to be hung on a branch when not in use. The pruner is a moveable blade working on an immovable part, similar to hand-pruners, on the end of a 4 ft. handle to which may be added 4 or 8 ft. extensions by means of a ferrule. The knife is kept open by a spring and closed by means of a cord.

Working from the top of the tree all dead wood, diseased tips, interfering branches and sucker growth are removed. If the tree is too dense it may be thinned out uniformly by pruning, care being taken that even pruning is done so that the tree is not patchy, and that all cuts are flush, i.e. no stubs left. Usually two men work together in large trees, so that by each man taking a side, there is no time lost in reclimbing. When the top work is done the climber lowers himself by pulling the tautline hitch and swinging outwards and downwards to the branch on which he is to work. As little weight as possible is put on the branch, but kept in the rope. The worker keeps himself from swinging into the trunk by placing his feet against lateral branches of the limb on which he is working. His body faces the trunk of the tree and the branches within reach are pruned, the branch upon which he is working left until last. All cuts greater than 1 in. diam. are painted with a black oil-base paint. Pole-saw cuts are painted by holding the brush between the pruner blades.

Cabling is done to support double trunks which are judged to be weak, or because of the susceptibility of the tree to wind damage, such as elms, and when a limb because of its weight and size may need supporting. The height the cable is to be is judged by the climber, the trunks are drawn together by means of a rope placed much higher than the cable is to be. A brace and bit are used to bore a hole in each of the trunks. Steel lags of a diameter slightly larger than the bit used are screwed into the holes in a direct line with each other. If a lag is off line the wood may split or the lag snap. A lag is similar to a hammock hook, having the hook closed. The measurement of the cable is given to the man on the ground who cuts the cable, allowing for the splice. The cable is six-strand steel wire about $\frac{1}{2}$ in. diam. Usually two men work on the cabling. One man loosens the strands on his end of the cable for about 8 in., then bends the strands back so that there will be no large loop when the splice is finished. The splice is done by putting the strands through the lag and winding one strand at a time tightly about the cable and the remaining wires, which must be held tightly against the cable or the splice will be bulky and insecure. Strong pliers are needed to draw the strands tightly, and leather gloves worn to prevent the hands being cut on the sharp wire. When one end is completed the same is done to the other, pulling the cable very taut before making the splice. When the final splice is made the ropes are released and the cable should be quite firm. In fine-limbed trees, such as birch, a very small clothes-line wire is used as cable with suitable eye-screws, very great care being taken not to draw the cable unnecessarily tight and not to split the branch by using too large a screw. The bark about the screw is then shellacked.

Cavity work necessitated by rotted stubs etc. is then done. To start the cavity a light mallet and a 1 in. chisel is used. The bark is first edged back till healthy living bark is reached, then shellacked. This is done in order to determine the extent of the cavity and also that no injury may be done to the live bark while the cavity is being cleaned out. Usually cavities in the upper tree and in branches are comparatively small, being seldom more than 2–3 ft. from top to bottom. The inside of the cavity is cleaned by means of flat chisels and gouge, until good solid wood is reached and the interior painted thoroughly with creosote. The chisel work is completed by cutting the opening of the cavity to a narrow oval, sharply pointed at top and bottom, in order to facilitate the healing over by new growth. The edges of the opening are made very definite, sharp, smooth, and receding. The bark is then cut back from the edge to about $\frac{1}{4}$ in. and shellacked. This is done in order that the cement is

brought tightly flush with the wood, while not interfering with the growth of, nor causing injury to, the bark.

Cement is thoroughly mixed with about 2–3 parts of sand to 1 of cement, with just enough water to moisten the mixture slightly, so that a minimum of contraction may be assured owing to cement shrinking on drying. The cement is then tamped into the cavity with the wooden end of the chisel to about 2–3 in. above the lower opening of the cavity, the cement protruding slightly. Four-ply roofing paper is cut to fit roughly the inside of the cavity and fitting tightly to the sides of the opening. It is marked at the point of contact with the edges of the cavity and cut directly across, and fitted into the cavity. Cement is tamped on the floor to a height of about 1 in. An upright is then put in which is about 3–4 in. high, a line is laid from edge to edge of the cavity and the upright marked. It is then cut from the mark to where it joins the opening at the floor. Cement is tightly tamped in on both sides of the upright, the face of the cavity being kept in place by the back of the hand. When the top of the upright is reached, another floor is put in, cutting the paper so that the edges and the upright are fitted perfectly. One upright is then put in on each side of the lower upright, that section completed; then a floor and one upright. The number and size of the uprights depends on the size of the cavity. Large cavities may require many uprights. The face is then 'slicked' or smoothed by a small flat spoon-shaped trowel, pressing all joinings tightly so that the paper protrudes about $\frac{1}{8}$ in. Care must be taken to scrape all cement from the $\frac{1}{2}$ in. of wood which was left between the bark and the cavity. This edge is again shellacked, and when the cement is quite dry the whole is painted black. The object of filling the cavity in this manner is to insure against the cracking of the cement, as well as for appearance. Quite large cavities, measuring as much as 20 ft. from top to bottom are not unusual. Usually much of the decayed wood in these cavities is soft and easily removed. When as much of this as can be conveniently reached is knocked out to make a tunnel up the tree, an oval-shaped opening is made to allow a circulation of air. Otherwise the smell of the decaying wood, together with the lack of air, would make it impossible to work in the tree. The cavity is treated in much the same way as a small cavity except that the tree is braced before the cement is put in. About $\frac{3}{4}$ in. holes are bored with a brace and bit on either side of the tree and a threaded steel rod is screwed through with the aid of a wrench. The superfluous length of rod is then cut off with a steel saw. The brace will give the most support if set about 1 ft. back from the opening of the cavity. In such a cavity there may be only 4–5 in. of solid wood left when all the decay is removed. It is then wise to put in several braces, perhaps three or four pairs about 4 ft. apart. When braces are put in pairs they lead from within 1 ft. of the opening of the cavity on each side, crossing each other to the opposite sides of the tree.

The large opening is completed as described for a small cavity. Cement at a mixture of about four to one is then poured in from the next opening, a little at a time, and tamped down carefully with a long-handled mallet. Care must be taken not to tamp too hard or the face of the large cavity is apt to crack, or one may have the misfortune to have the cement fall out altogether.

A trick some builders have in Toronto is to take some worthless land, level it off by filling in the hollows with ashes and tin cans, and finishing with 1 ft. or so of good earth and turves. The result is very nice, except that shortly the leaves of the trees will start to turn brown and the proud owners of the new estate, who were influenced in the purchase to a great extent by the appearance of the lawns and trees, appeal to a tree surgery company for advice. The reason for the weakening of the trees is that the level of the ground about the tree has been changed: the bark about the tree rots and a flow of sap is prevented. If caught in time the condition may be remedied by removing the earth from around the tree to about 3 ft. and down as far as the original ground level, the sides being supported by a wall of stone-work.

Tiling is a somewhat lengthy procedure but may be considered as being worth the trouble and expense by the ease with which a tree may be watered and fertilized when necessary. A trench, which is wide enough for a man to work in conveniently, is dug around the tree at approximately the same distance as the extension of the branches. This may not be possible because of adjoining properties or sidewalks and roads in the way: in such cases the distance must be governed by what is possible. The trench is dug down to the roots, care being taken that sufficient soil covers the roots so that they are not injured. Another trench is dug from the tree in a direct line to the original trench. The bottom of the trench leading from the tree will, naturally, be a decline, because at the tree it will be very shallow, while at the other end it will be the same depth as the main trench. Terra-cotta tiles about 4 in. diam. are then laid in the main trench. Where each tile joins the last a small piece of roofing paper covers the join. The tile which makes the T-join that starts the tiling up to the tree

is made to fit by breaking the sides of the ends of the three adjoining tiles so that they fit reasonably well, and the fit made perfect by the use of cement. The final tile at the tree is fitted in perpendicularly and is flush with the ground. The complete trench is then filled in solidly, and sodded if the tree is on a lawn. The feeder-pipe is cemented at ground level and covered when not in use. Fertilizer may be poured into the feed-pipe and washed round the tree with water by means of the tiling system. In Toronto where a long dry period is not uncommon, tiling has been the only means of insuring sufficient moisture to many valuable trees.

Spraying is one of the most important jobs a tree surgeon has to do, and to do it intelligently he should have a working knowledge of entomology and mycology. Unfortunately the opportunity of studying these subjects is not often available to the tree surgeon and he must glean from those who give reluctantly from a meagre store of knowledge. Different localities have different pests, and therefore the tree surgeon's job is simplified by knowing those which affect the trees in the particular locality in which he lives. Aphides, tent caterpillars, and scales, particularly oyster shell, are the greatest enemies of Toronto's trees. The American elm, which is by far our largest shade tree in Ontario, is greatly affected by caterpillars and it is here that the power sprayer, which is capable of throwing a spray up to 70 ft., is used. One must be very careful, however, not to break small branches by allowing the spray jet to come directly in contact with them. Usually such large trees must be climbed and the pressure considerably lessened so that the tree may be well sprayed without danger of breakage. Before spraying one must see that all woodwork or stonework with which the spray may come in contact, is well covered because of stains which the spray is apt to leave. Pools which contain fish must also be protected because of the effect of the poison, especially arsenicals and derris extracts. Small trees may be sprayed by the use of a hand-sprayer, of which there are many types; a common one being a knapsack sprayer. The tank is air-compressed before the operator climbs the tree.

So much has been written about spraying that there is little to be said here concerning this subject: the only reason it is mentioned is to show that it is part and parcel of the tree-surgeon's work.

At the present time there is only one way in which one may learn tree surgery, and that is to be employed by a tree-surgery company. One's first task is that of 'brush hauler', which consists of clearing away the prunings and seeing they are burnt, doing the inside work on large cavities which extend from the ground, passing up tools for the climbers, and doing odd jobs, many of which are unpleasant. After a time one is allowed to climb small trees, learn to use a rope, learn the names of trees, etc. Eventually one is allowed to climb high or dangerous trees, and finally to do tree surgery and supervise jobs. However, not all men who profess to do tree surgery have had such training. There have been cases of spraying being done with plain water by people who, when they did not know what to do, were not conscientious enough to find out, and felt they must make some kind of show to collect their money. Tree surgery is still fairly new as a profession and hence there is no formal education provided by the government. Anyone is at liberty to pose as a tree surgeon and, though knowing nothing, may bluff his way quite successfully.

Tree surgery is a luxury trade. The cost of labour is far too high, as a rule, for the average person. Yet all trees, no matter to whom they belong, are the concern of everyone. It is in the interest of the rich and the poor man alike to see that all trees, whether privately or publicly owned, are kept in a healthy condition. In my opinion tree surgery should be, if not a national concern, at least one for the various municipalities individually. The cost of labour, material, tools, maintenance, and administration could be covered by the charges made to those wishing to have such work done. This is actually the case, I believe, in the City of Westmount, Montreal. In Toronto there is a Forestry Department which tends the trees belonging to the city but not those privately owned. In England tree surgery is not, so far as I know, carried out to any extent at the present time, and there could very easily be arranged courses for the tuition of tree surgeons. If such work is to be done by the government only certified graduates from these schools should be employed. In any case the skilled tree surgeon would be protected if only those bearing certificates of qualification were allowed to do this work.

REPORT OF THE COUNCIL OF THE ASSOCIATION OF APPLIED BIOLOGISTS FOR THE YEAR 1941

THE Officers and Council of the Association were as follows:

President: H. MARTIN, D.Sc.

Vice-Presidents: Miss W. E. BRENCHLEY, D.Sc., F. R. PETHERBRIDGE, M.A.

Hon. Treasurer: W. C. MOORE, M.A.

Hon. Secretaries: General and Botanical: W. P. K. FINDLAY, D.Sc.; Zoological: H. F. BARNES, M.A., Ph.D.

Hon. Editors of Annals of Applied Biology: General and Botanical: Prof. W. B. BRIERLEY, D.Sc.; Zoological: C. T. GIMINGHAM, B.Sc., F.I.C.

Council: G. C. AINSWORTH, Ph.D., G. E. BLACKMAN, M.A., *D. E. GREEN, M.Sc., R. V. HARRIS, B.Sc., *W. E. H. HODSON, D.I.C., H. G. H. KEARNS, Ph.D., O. B. LEAN, B.Sc., *C. POTTER, Ph.D., *E. A. PARKIN, Ph.D., W. M. WARE, D.Sc.

In spite of war-time difficulties the Association has had a very successful year, all the meetings being well attended, the attendance at the ordinary meetings being on the average 36 Members and 14 visitors. It is with much regret that the Association has to record the deaths of Sir Arthur Hill who had been a Member for over 20 years, of Mr Stafford Allen and of Dr R. P. Hobson. Twenty-three new Members were elected and the Association now numbers, as far as can be ascertained, 312 Members including the 12 Honorary Members, and the active membership is probably greater than it has ever been. Of the ordinary Members 36 are known to be resident abroad.

On 25 July, 25 Members visited the John Innes Horticultural Institution at the kind invitation of the Director who arranged for a very interesting series of demonstrations to be shown.

The following papers and discussions were presented to the Association during the year 1941:

14 Feb. J. MACLEOD: 'Recent work on the sheep tick and its bearing on control measures.' R. P. HOBSON: 'Recent work on the sheep maggot problem.' I. THOMAS: 'Some practical aspects of the sheep blowfly problem.'

28 Mar. A joint discussion was held with the Society for Experimental Biology on 'The relationship between pure and applied biology.'†

Prof. P. A. BUXTON gave the opening address and Prof. W. B. BRIERLEY followed. Dr V. B. WIGGLESWORTH spoke on 'Malaria and war', and Dr M. A. H. TINCKER gave a paper on 'Aspects of the practical application of growth substances to practice.'

10 Oct. *Symposium on wireworm investigations.*

J. C. F. FRYER: 'Investigations on wireworms: introductory.' S. G. JARY: 'Wireworms and crop production.' G. SALT and F. S. J. HOLLICK: 'The analysis of wireworm populations.' F. YATES and D. J. FINNEY: 'Statistical problems in field sampling for wireworms.' A. C. EVANS and H. C. GOUGH: 'Observations on some factors influencing growth in wireworms of the genus *Agriotes* Esch.' H. W. MILES: 'Wireworms and agriculture, with special reference to *Agriotes obscurus* L.' M. COHEN: 'Observations on the biology of *Agriotes obscurus* L.'

5 Dec. *Discussion on some problems in war-time horticulture with particular reference to vegetable production.*

H. V. TAYLOR: 'Problems in vegetable production: introductory.' W. F. BEWLEY: 'Some problems of the glasshouse industry in war time.' T. WALLACE: 'Some aspects of the nutrition of vegetable crops.' G. E. BLACKMAN: 'The control of weeds in onions.'

CHESTER COOK: 'Some aspects of tree surgery in Canada.'

* New members for 1941.

† See *Nature*, Lond., **147**, No. 3728, 12 Apr. 1941.

On 28 Mar., 10 Oct. and 5 Dec., the Association was kindly allowed the use of the lecture theatre at the London School of Hygiene and Tropical Medicine, and the Council is glad to have this opportunity of putting on record its sincere appreciation of the hospitality afforded by the authorities of the School of Hygiene.

The joint committee set up in collaboration with the British Mycological Society to develop a system for recommending the use of proprietary insecticides and fungicides held a further meeting on 9 May after the agreed report had been submitted to the Ministry of Agriculture and Fisheries and to the Association of British Insecticide Manufacturers. While the scheme proposed has not been accepted, the Council understands that a simplified scheme has been prepared by the Ministry and is now under discussion. The development of this official scheme has no doubt been due partly to the activity of the joint committee.

As a result of the resolution passed by the General Meeting on 6 Dec. 1940, a small committee was set up to investigate means of bringing to the notice of the authorities the pressing problems in applied biology which have arisen as a result of the war. The British Ecological Society and the Society for Experimental Biology had formed similar subcommittees with the same object in view, and a joint committee of the three societies was therefore formed. This committee has been enlarged at the request of a joint committee of the Agricultural Research Council, the Department of Scientific and Industrial Research and the Medical Research Council into the Biology War Committee which has been entrusted with the work of bringing to the notice of the Research Councils, problems in applied biology which are deemed to be of national importance.* This Biology War Committee has held a number of meetings and has already been able to get various projects under way.

The Association was able to continue the publication of the *Annals of Applied Biology* although difficulties of supply made impossible the maintenance of the original texture and quality of the paper. In view of the high and steeply rising costs of production it is *imperative* that authors should condense their manuscripts, delete every non-essential word and illustration, and reduce proof corrections to a minimum. It would greatly assist the Hon. Editors if intending contributors would complete their manuscripts in accordance with the 'Notice to Contributors' on the back cover of the *Annals*, and if they would consult the last published issue of the *Annals* for details of format.

Vol. **28** (1941) comprised pp. ix + 416 and 14 plates as against pp. x + 576 and 22 plates for vol. **27**. Including papers published as 'Proceedings' vol. **28** comprised 44 papers and 24 reviews, 39 of the former being by Members of the Association. The papers may roughly be classified as follows: general applied botany, 5; mycology and fungal diseases, 11; bacterial diseases, 1; viruses and virus diseases, 4; entomology and insect pests, 13; plant protection, insecticides and fungicides, 1; helminthology and nematode diseases, 4; applied microbiology, 4; general, 1.

The several issues of the *Annals of Applied Biology* for 1941 were published on the following dates: No. 1, 10 March; No. 2, 6 May; No. 3, 26 August; No. 4, 4 December.

* See *Nature*, Lond., **149**, No. 3774, 28 Feb. 1942.

REPORT OF THE HON. TREASURER FOR THE YEAR ENDED 31 DECEMBER 1941

During the year ended 31 December 1941, subscriptions and entrance fees, including arrears, amounted to £343. 10*s*. 6*d*., which was £69. 6*s*. 6*d*. less than the previous year. Current subscriptions were down by only £12. 10*s*. 0*d*., and the decrease was mainly due to the fact that in 1940 an exceptionally large amount of arrears was collected. Moreover, no sum has been included in the total, as has been done in previous years, for subscriptions not yet paid but considered good. These arrears amount to about £20.

Income from the sale of the current volume of the *Annals of Applied Biology*, and from reprints, amounted to £579. 14*s*. 2*d*., a decrease of £126. 12*s*. 2*d*., of which more than half was due to a falling off in the sale of reprints. There was also a very noticeable fall in the sales of back volumes. The size of the *Annals* was 416 pages compared with 576 pages in 1940, and the cost of producing it dropped to £810, a decrease of £244. 10*s*. 3*d*. on last year's figure.

The income for the year exceeded the expenditure by £117. 11*s*. 11*d*. and after all obligations have been met, the assets of the Association amount to £928. 9*s*. 3*d*., of which £530. 10*s*. 3*d*. is represented by National Savings Certificates, and £200. 17*s*. 0*d*. by the estimated value of the stocks of the *Annals*. The financial position of the Association is therefore quite sound.

On behalf of the Association, I acknowledge with gratitude and warm appreciation the receipt in May of a sum of £50, allocated to the Association by the Council of the Royal Society, from a special Rockefeller Foundation Grant in aid of Scientific Publications. Certain steps had necessarily been taken to curtail expenditure on the 1941 volume of the *Annals* before this grant was made, and its receipt not only relieved any anxiety that had been felt, but also permitted some expansion of the August and November issues of the *Annals*. It was subsequently thought expedient to earmark the £50 for the purpose of maintaining and, if possible, increasing the size of the *Annals* in 1942, a decision that appears to be fully justified in view of the now impending increase of 20% in printing costs.

W. C. MOORE
Hon. Treasurer

THE ASSOCIATION OF APPLIED BIOLOGISTS

Dr. *ANNALS OF APPLIED BIOLOGY*, INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1941 *Cr.*

Expenditure	£ s. d.	Income	£ s. d.
To Estimated Value of Stock, 1 January 1941	183 17 0	By Sales—Current Volume	521 3 8
To Cambridge University Press	810 0 0	By Sales—Back Volumes, Parts and Sets	14 12 2
		By Sales of Reprints	58 10 6
		By Estimated Value of Stock, 31 December 1941	200 17 0
		By Balance, carried down	198 13 8
	£993 17 0		£993 17 0

Dr. GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1941 *Cr.*

Expenditure	£ s. d.	Income	£ s. d.
To *Annals of Applied Biology* balance brought down	198 13 8	By Members' Subscriptions	332 10 0
To Printing and Stationery	6 1 8	By Entrance Fees	11 0 6
To Postages and Cheque Stamps	11 12 2	By Interest on National Savings Certificates	15 1 0
To Subscription—Parliamentary Science Committee	5 5 0	By Interest on Bank Deposit Account	9 1
To Sundry Out-of-Pocket Expenses of Editors, Secretaries and Treasurer	15 12 2		
To Audit Fee	4 4 0		
To Balance, being Excess of Income over Expenditure for the year	117 11 11		
	£359 0 7		£359 0 7

BALANCE SHEET, 31 DECEMBER 1941

Liabilities and Surplus	£ s. d.	£ s. d	Assets	£ s. d.	£ s. d.
Sundry Creditors:			Cash at Bank:		
Cambridge University Press	215 13 8		Current Account	277 0 11	
Audit Fee Reserve	4 4 0		Deposit Account	176 0 0	
Sundry Expenses	16 9 3				453 0 11
		236 6 11	301 National Savings Certificates		530 10 3
Subscriptions and Entrance Fees, paid in advance		19 12 0	Stock of *Annals of Applied Biology* at estimated value		200 17 0
Excess of Assets over Liabilities:					
As Balance Sheet of 31 December 1940	760 17 4				
Add: Balance of Income and Expenditure Account for 1941	117 11 11				
Rockefeller Foundation Grant (per Royal Society)	50 0 0				
		928 9 3			
		£1184 8 2			£1184 8 2

W. C. MOORE, *Hon. Treasurer*

We certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to us, and, in our opinion, the Balance Sheet exhibits a true and correct view of the state of the affairs of the Association according to such books, vouchers and documents.

(Signed)
H. J. COX & CO.
Incorporated Accountants
Auditors

HARPENDEN, 7 *February* 1942